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SALMONELLA INFECTIONS IN DUCKS

OBSERVATIONS ON THE VALUE OF THE AGGLUTINATION TEST IN THE ERADICATION OF INFECTION AND INVESTIGATIONS ON THE CYCLE OF INFECTION *via* THE EGG

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INTRODUCTION

Salmonella infections in domesticated birds are not rare, even if one excludes *S. pullorum* and *S. gallinarum*. During the past 20 years frequent references have been made to outbreaks of disease caused by *S. typhi-murium*, *S. enteritidis* (Gaertner) and *S. anatum*. References to the isolation of these organisms from different species of domesticated birds are tabulated in a previous paper (Garside and Gordon, 1940).

These infections assume added importance through the possibility of " food poisoning " arising in human beings from the consumption of infected duck eggs. Scott (1930) describes three and mentions seven outbreaks of food poisoning in man caused by *S. typhi-murium*, in which he believed the consumption of duck eggs to be the source of infection. Warrack and Dalling (1932, 1933) showed that infection of duck eggs does occur, and that one source of infection is the infected ovary, while Beller and Reinhardt (1934) found that of 1,506 eggs examined nearly 1 per cent. were infected with organisms of the paratyphoid group. Wanner (1937) reported a case of *S. enteritidis* Breslau (*S. typhi-murium*) in a man following the consumption of a duck egg, and on examination of 376 eggs laid by ducks on the farm in question, *S. enteritidis* Breslau was isolated from 19; 60 per cent. of the ducks on the farm showed ovarian degeneration and *S. enteritidis* Breslau was isolated from the ovaries of 19. De Koning (1936) described an outbreak of food poisoning in 60 people from the consumption of ice cream containing raw duck eggs. Twenty-two of the 25 ducks which produced the eggs reacted positively to the agglutination test for *S. typhi-murium*, and in studying the same outbreak Jansen (1936) isolated identical strains of *S. typhi-murium* from the patients' faeces, from the yolks of eggs, and from the degenerate ovaries of the reactor ducks. Hedstrom (1941) reported an outbreak of *S. typhi-murium* infection in fowls, turkeys, and geese on the same farm, which occurred simultaneously with an illness in the owner's family in which identical strains of the organism were recovered. Finally, Muller (1941) recorded 23 cases of food poisoning admitted to a Hamburg hospital each with the history of having eaten raw duck eggs. *S. typhi-murium* was isolated from 18 of the cases and *S. enteritidis* from two.

In their previous publication Garside and Gordon (1940), described an extensive outbreak of salmonellosis in ducklings. The paper dealt generally with the history of the outbreak, the symptoms

and *post mortem* lesions in affected ducklings, and the efficacy of the control measures adopted. This present paper is an elaboration of the previous report, and is concerned with the elimination of carriers by means of the agglutination test and the isolation of the causal organisms from reacting ducks, and from eggs laid by these ducks. The correlation between the serum titre of the reacting ducks and the activity of their ovaries, together with the recovery of the organisms from these organs, is also discussed and an account is given of two breeding experiments.

PRESENT INVESTIGATION

(1) *Blood Testing on the Farm*

The losses in this outbreak, which were shown to be due to infection with *S. enteritidis* and *S. typhi-murium*, were very severe, amounting to 1,000 ducklings a day with a total death-rate of 30 per cent. in a flock of 57,000 ducklings. Hygienic measures were successful in reducing the mortality to below 3 per cent. In view, however, of the references in the literature already cited to the recovery of the above organisms from duck eggs and to the detection of adult carriers by means of the agglutination test (Scott, 1930, 1932; Warrack and Dalling, 1932, 1933; De Koning, 1936 and Van Oijen, 1940) total eradication could not be reasonably anticipated if adult carriers were allowed to remain in the breeding flock. Accordingly the entire breeding flock was tested, and since the results obtained were not in accordance with expectations, a description is given of the method of testing and of the blood testing records.

The sera were tested in the first place against two antigens composed respectively of six strains of *S. enteritidis* and six strains of *S. typhi-murium*. The tests were carried out separately, but simultaneously, in serum dilutions of 1/20 and 1/50. The mixtures were incubated in a closed water bath at 56° C. for two hours, and were afterwards allowed to stand at room temperature for several hours before reading. The broth cultures of the respective organisms used as antigens were killed by two methods, namely, by the addition of 0·25 per cent. formalin, and chloroform. Little difference could be found in the efficacy of the two methods. The formalised antigen gave slightly more clear cut results with low titre reactors, and was used throughout the bulk of the testing. Later, homologous antigens were used, prepared in the same way from six strains of *S. enteritidis* and six strains of *S. typhi-murium* recovered from the outbreak. When reading the tests any trace of agglutination in the 1/20 serum dilution, even though the supernatant fluid was not clear, was taken as evidence of infection. Both authors had had previous experience in testing small numbers of ducks for Salmonella infection, and were agreed that in the elimination of such infections it was necessary to take cognisance of even the slightest reaction in the lowest dilution. All reacting samples were retested as a check on the result, and the usual serum and antigen control tests were performed daily.

At the initial test approximately 21,000 ducks were tested and 978, or 4·6 per cent., gave a positive reaction to one or other of

the antigens, in the proportion of four *S. enteritidis* reactors to one *S. typhi-murium* reactor. Two re-tests of the flock were subsequently carried out at intervals of two to three months. At the second test 18,500 ducks were tested and 5·9 per cent. reactors were disclosed; at the third test 10,400 ducks were tested, with 4·4 per cent. reactors. After each test the reacting birds were removed from the pens, but were not removed from the farm, the majority being placed in a series of large pens which had been "resting" for some time. These birds were not tested at the second and third tests, only birds which had previously passed the test being tested on these occasions.

This failure of measures based on diagnosis by blood testing to reduce the number of carriers was tentatively ascribed to the non-removal of reactors, and to ineffective hygienic measures. Later investigations at the laboratory showed that other factors such as the fluctuation in titre of the serum of carrier birds and the influence of ovarian activity could be, and were, involved. The main cause of the continued prevalence of reactors, however, would appear to be the reinfection of the ducks which took place continuously when they reached maturity, and not the continuance through life of an infection contracted as ducklings. This view finds support by comparing the distribution of reactors in the various age groups, when it becomes noticeable that the percentage number of reactors increases with the age of the ducks. For example, of the one-year-old ducks only 1·3 per cent. reacted, of the two-year-olds 4·8 per cent. reacted, of the three-year-old birds 9·8 per cent. reacted, and of the four-year-old and older birds 17·3 per cent. reacted.

(2) *Experimental Work*

To check the accuracy of the agglutination test, ascertain whether a reaction indicated present infection and corroborate our inference that a response at 1/20 was evidence of infection, 113 reacting ducks picked at random from the three- and four-year-old birds were brought to the laboratory. To minimise risks of infection, they were segregated in pairs, each pair occupying a wire floored metal coop, placed on trestles 3 feet above the floor. The ducks were then periodically tested and killed for bacteriological examination as time permitted. Dead birds were examined immediately. Whenever possible, blood samples were taken for testing before killing. Eighteen months elapsed before all the birds were examined. Some were tested up to eight times at intervals varying from one to seven months. The results of these tests are shown in Table I.

(3) *Bacteriological Examinations*

Cultures were made into nutrient broth and on to agar slopes from the heart blood, liver, spleen, gall bladder wall, and from any lesion such as inspissated or encapsulated egg material. Direct cultures were made in the same way from ova in the visually affected part of any ovary. The remainder of the ovary was then dissected

TABLE I

POSITIVE BACTERIOLOGICAL RESULTS

Duck No.	*Farm Test**	*Laboratory Tests*						*Macroscopic appearance of ovary at autopsy*	*Organism recovered from*
	1st	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*		
321	1/50	1/50 K.						N.I.	Ovary
721	1/50	1/100 K.						N.I.	Ovary
315	1/50	1/10000 K.						D.I.	Ovary
110	1/20	1/100 D.						D.I.	Ovary
900	1/20	0 D.						N.I.	Ovary
262	1/50	1/100 D.						N.I.	Ovary
402	1/50	1/100 D.						N.I.	Liver and spleen
808	1/20	1/20 D.						N.I.	Ovary
342	1/20	1/20 K.						N.I.	Ovary
304	1/50	1/50 K.						N.I.	Encapsulated egg
408	1/50	1/1000 K.						D.I.	Ovary
620	1/50	1/50 K.						N.I.	Ovary
540	1/50	1/50 D.						N.I.	Ovary
967	1/50	1/1000 K.						N.A.	Ovary
960	1/20	0 K.						D.I.	Ovary
76	1/50	0 K.						N.I.	Ovary
223	1/50	1/1000 K.						D.I.	Ovary and intestine
866	1/20	0	0 K.					D.I.	Ovary
781	1/50	1/50	0 K.					D.I.	Ovary
406	1/50	1/50	1/50 K.					D.I.	Ovary
21	1/50	1/50	1/50 K.					N.I.	Ovary
820	1/50	1/100	1/100 D.					N.I.	Ovary
770	1/50	1/100	1/100 D.					N.I.	Ovary
809	1/50	1/20	1/100 D.					D.I.	Ovary, heart blood, one encapsulated egg
607	1/50	1/200	1/1000 D.					D.I.	Ovary, spleen, gall bladder, heart
911	1/50	1/200	1/100 D.					D.I.	Encapsulated egg, gall bladder

Salmonella enteritidis reactors

									S. enteritidis recovered
308	1/50	1/50	1/50	0 K.				D.I.	Ovary
148	1/50	1/20	1/20	0 K.				N.I.	Ovary and encapsulated egg
226	1/50	1/50	1/20	1/50 K.				D.I.	Ovary
320	1/50	0	0	0 K.				D.I.	Ovary
280	1/50	0	0	0 D.				D.I.	Ovary
181	1/50	1/1000	1/100	1/500 D.				N.I.	Intestine and encapsulated egg
384	1/50	1/50	1/100	1/100 K.				N.I.	Ovary
443	1/20	1/20	0	0 K.				N.I.	Ovary
75	1/50	1/50	1/100	0	1/20 K.			N.I.	Encapsulated egg
522	1/50	1/50	1/100	1/20	1/20 K.			N.I.	Ovary and encapsulated egg
382	1/50	1/50	1/100	0	0 K.			N.I.	Encapsulated egg
138	1/50	1/20	1/50	0	0 K.			D.I.	Ovary
689	1/50	1/100	1/200	1/50	1/50 K.			D.I.	Ovary
409	1/50	1/100	1/20	0	1/20 K.			N.I.	Encapsulated egg
459	1/50	1/50	1/100	1/50	1/50 K.			N.I.	Ovary
468	1/50	1/20	0	0	0	1/50	1/50 K.	D.A.	Ovary
478	1/50	1/50	1/50	0	1/20	1/50	1/20 K.	D.A.	Ovary
112	1/20	1/20	1/20	0	0	0	0 K.	N.I.	Ovary
276	1/50	1/100	1/20	0	0	0	1/50 K.	D.I.	Ovary
338	1/50	1/100	1/20	0	0	0	1/50 K.	N.A.	Ovary
									S. typhi-murium reactors : *S. typhi - murium* recovered.
915	1/50	1/100 K.						N.I.	Ovary
346	1/50	1/1000 K.						N.I.	Ovary
106	1/50	1/100 K.						D.A.	Ovary
353	1/50	1/100 K.						D.A.	Gall bladder
675	1/20	0	1/50 K.					N.I.	Gall bladder
85	1/50	1/200	1/100	1/200	1/50	1/20 K.		Drake	Gall bladder

D = Died.
K = Killed.
N.I. = Normal inactive
D.I. = Degenerate inactive.
N.A. = Normal active.
D.A. = Degenerate active.
* For the first test the dilution of 1/50 was the highest used.

out, minced sterilely, and the pulp filtered through sterile glass wool into 100 c.c. of peptone broth containing brilliant green in final concentration of 1 in 75,000. Ovaries that seemed normal were similarly treated. When the ovary was active, however, and included many large ova, these were discarded because the action of the brilliant green was likely to be interfered with by the much increased bulk. The small intestine contents squeezed into a funnel were filtered through sterile glass wool into similar culture flasks. After incubation overnight, material was taken from these flasks for inoculation on MacConkey plates. Subsequently, non-lactose-fermenting colonies were picked off for identification.

Cultural and Serological Reactions of the Organisms Isolated.—Five representative strains of *S. typhi-murium*, together with 18 strains of *S. enteritidis*, were isolated from these ducks and retained for fuller examination. All were motile, gram-negative and were tested with the same results on three separate occasions.

Fermentation tests were carried out in peptone water containing 1 per cent. of the test substrate and Andrade's indicator; other media were prepared according to their original formulae.

A. *S. enteritidis.*—Acid and gas were produced constantly in dextrose, maltose, arabinose, mannitol, dextrin, xylose and dulcitol. The fermentation of dulcitol, although constant, was always delayed until the third or fourth day of incubation. Sorbitol was fermented by all strains except three. No change was observed in lactose, saccharose, inositol, and salicin. Litmus milk was made constantly alkaline; all strains produced H_2S; indol was not produced. Tested in Stern's glycerol medium (Stern, 1916), all gave positive reactions, and by the method of Bitter, Weigmann and Habs (1926) using rhamnose, arabinose and dulcitol as the test substrates, all fermented rhamnose, but not arabinose and dulcitol.

Serologically, each strain, which had been previously identified by agglutination with a standard "H" specific agglutinating serum (Standards Laboratory, Oxford), was examined more exhaustively. "H" specific agglutinating sera, using formalised young broth cultures, were prepared against six of the 18 strains and against two type cultures of *S. enteritidis* Gaertner. All 18 strains were agglutinated by each serum either to titre or almost to titre, and by absorption tests all were found capable of completely removing the specific agglutinins from each serum, including the sera prepared against the type cultures. Reciprocally, the two type cultures completely removed the agglutinins from the six representative sera. The strains were therefore identified as *S. enteritidis*, no antigenic difference in any of the 18 strains being observed; but from their reactions in Bitter's medium they all appeared to belong to the Essen variety (Bergey, 1939).

B. *S. typhi-murium.*—Five strains of this organism isolated from the reacting ducks were likewise examined. In carbohydrate media, acid and gas were produced by all in glucose, maltose, arabinose,

mannitol, dulcitol, sorbitol, dextrin and xylose. Inositol was fermented by all strains except one. No change was observed in lactose, saccharose and salicin. All produced H_2S, and alkalinity in litmus milk but failed to produce indol. In Stern's glycerol medium all gave positive reactions. All failed to ferment Bitter's rhamnose medium. All but one strain broke down sodium dextrotartrate medium (Brown, Duncan and Henry, 1924). Serologically, preliminary agglutination tests with " H " specific serum (Standards Laboratory, Oxford) had identified them as *S. typhi-murium*. " H "-agglutinating sera prepared against each of the five strains, and against a type culture of *S. typhi-murium* cross-agglutinated with the other four strains to titre or almost to titre ; absorption tests showed each strain to be capable of removing the specific agglutinins from each of the five sera, and also from the serum prepared against the type culture. Reciprocally, the type culture removed the agglutinins from the sera prepared against the five duck strains. As no difference in antigenic structure could be demonstrated, all five strains were concluded to be *S. typhi-murium*.

(4) *Results of Post-mortem and Bacteriological Examinations*

The causal organisms were recovered from 55, or 48 per cent., of the 113 ducks examined ; the relevant details of the bacteriological findings are shown in Table I. To demonstrate the association of these findings with the serum titres and the activity of the ovary, the blood testing results and *post-mortem* appearances of this organ are also shown. This table is concerned only with birds in which positive bacteriological results were obtained.

Eight birds of the 113 lost their rings during the course of the investigation. Three of the birds whose rings were lost were in the group with positive bacteriological findings and, their records being incomplete, are not included in the tables. The remaining 58 birds gave negative bacteriological results. Space does not allow the recording of complete details of these birds, but 18 of them showed lesions in the ovary at autopsy, and it is interesting to note that seven of these birds had previously recorded a serum titre of 1/100 or higher when blood tested. The ovaries of the remaining 40 birds were normal to naked eye examination and their blood testing records did not show a titre higher than 1/100, which in effect was reached by only five of these birds.

Table I shows clearly the marked fluctuations in serum titre which occurred amongst the birds, to which reference will be made later, and also draws attention to the frequency with which the organisms were isolated from the ovary. This organ was found to be infected in 45 out of the 55 birds which gave positive bacteriological results, and to be the sole organ infected in 38. Other sites from which the organisms were recovered comprised : old encapsulated egg concretions, ten times, and in six of these this was the only site from which the organisms were isolated ; gall bladder

wall four, in three of which this organ was the only site affected; intestines and heart blood twice; liver and spleen twice; and liver alone once. In one bird the liver and spleen were the only organs found affected.

In assessing the possibility of infections being transmitted by these reacting ducks, it is of interest that the ovary is the organ most often infected; resulting transmission of the organisms through the egg is of significance from the public health standpoint as well as in the infection of ducklings.

From our previous observations the reinfection of adults seemed to explain the persistence of infection. However, the results obtained with the intestine, which is the source of origin of infection in the transmission amongst adults, were singularly negative. The organisms were only recovered twice from the intestines, but infection was demonstrated in the gall bladder wall in five birds, from which site transmission *via* the droppings would undoubtedly occur. An improved technique might result, it is appreciated, in a higher percentage recovery of the organisms from the intestines. This would indicate better whether carrier ducks themselves are capable of perpetuating infection in the adult flock, or whether other hosts, such as rats and mice, must be considered the more important. At present, the latter seems the more probable.

CORRELATION OF RESPONSE TO THE AGGLUTINATION TEST, AND OVARIAN ACTIVITY

Attention has already been drawn to the considerable fluctuation in the response given by the birds at the various blood tests.

Forty-three ducks which originally reacted to the test showed a negative reaction at some time or other, and 17 of these at a subsequent test gave a positive agglutination again. Of these 43 fluctuating reactors, 20 were found to be infected, and 13 were actually negative at the last test before death.

That 20 birds in a total of 113 (17·7 per cent.) gave successively positive and negative results, and were definitely shown to be infected, is of the highest significance in the eradication of carriers by blood testing. The fluctuation indicates that a single blood test is valueless, for as many as 17 per cent. of birds that are actually infected may be left in the flock.

This fluctuation appears to be associated with the activity of the ovary and receives corroboration from the blood testing records of the birds in the two breeding pens (Table III). While there was no significant fluctuation in the titre during the first laying period at the laboratory, there was a definite decrease in the titre of ten out of the 16 ducks when egg production ceased (fifth test), six failing to show any reaction (not including those which showed consistent negative results). When the second laying period began (seventh test) there was a general rise in the titre, and of the eleven birds which were negative three months earlier, five now gave positive reactions.

CORRELATION OF OVARIAN ACTIVITY AND BACTERIOLOGICAL FINDINGS

In Table II an attempt is made to clarify the results and correlate the isolation of the organisms from the ovary with its activity and pathological condition. The co-operation of these two factors must be held to account for the passage of infection through the egg. It will be seen that 67 of the ducks showed normal ovaries, of which 14 were active and 53 inactive ; the organisms were recovered from two of the 14 active and 19 of the 53 inactive normal ovaries. The 38 remaining reactors showed ovarian degeneration evinced by angular, stalked, greenish or olive green ova, similar to those seen in Pullorum disease of fowls. Of these ovaries 12 were functioning and the organisms were recovered from three ; 26 ovaries were inactive and the organisms were recovered from 18. Although 42 birds showed infection of the ovary, only five, *i.e.*, those with active ovaries (normal or pathological) were capable of transmitting infection through the egg at the time of the examinations. The remaining 37 must be considered potential transmitters when, and if, the ovaries become active. The presence of infection in the encapsulated egg concretions which were found either free in the peritoneal cavity or attached to the peritoneum suggested that on occasion the infected ova were shed into the peritoneal cavity. In six of the ten cases in which the organisms were isolated from this site the ovary was negative ; this would seem to indicate that infection had previously been present in that organ.

As one would anticipate, the organisms were isolated in substantially higher percentage from ovaries showing pathological changes, but it is worthy of note that over 31 per cent. of macroscopically normal ovaries revealed the presence of infection.

Correlation of Serum Titre and Bacteriological Findings

As the titre of the serum rose the organisms were isolated with greater frequency. Out of 40 birds with a titre of 1/100 or higher, 27, or 67·5 per cent., were positive bacteriologically ; of the remaining 65 birds, with a titre below 1/100, 25, or 38·5 per cent., gave positive results. Although in some cases the inability to recover the organism from 58 out of 113 ducks examined may have been due to failure to examine the site of infection, the results would indicate that birds with a titre of 1/100 or higher were suffering from present infection. This is further supported by the finding that 18 of those from which the organisms were recovered showed a titre of 1/100 or greater at the time of death, while our other records show that only four of the 53 birds not shown to be infected had a titre higher than 1/50 at the time of death. It is possible then that titres of 1/50 and under refer to birds recovering from past infection.

On the other hand, of 13 birds whose sera did not agglutinate

TABLE II

CONDITION OF OVARY AT AUTOPSY CORRELATED WITH THE ISOLATION OF THE ORGANISMS AND THE HIGHEST SERUM TITRE

Titre	*Number of birds*	*Birds found infected*	*Normal Ovaries*				*Pathological Ovaries*				*Infected in sites other than ovary*
			Active	*Organisms present*	*Inactive*	*Organisms present*	*Active*	*Organisms present*	*Inactive*	*Organisms present*	
1/20	13	7	1	0	9	5	2	0	1	2	0
1/50	52	18	9	0	27	6	6	2	12	7	3
1/100	22	17	1	1	15	8	2	1	3	3	4
1/200	6	3	2	0	0	0	1	0	3	1	2
1/500	2	0	0	0	1	0	0	0	1	0	0
1/1000	8	6	1	1	1	0	0	0	5	4	1
1/5000	1	0	0	0	0	0	1	0	0	0	0
1/10000	1	1	0	0	0	0	0	0	1	1	0
	105		14		53		12		26		
		52		2		19		3		18	10

Active : indicates that the bird was laying.
Inactive : indicates that the bird was not laying.

at any test in a dilution higher than 1/20, seven were shown to be infected, and the organism was recovered from the ovaries. This is a point of importance with a bearing on routine blood testing. It justifies our insistence that birds showing a reaction in a serum dilution of 1/20 should be regarded as potential sources of infection and removed from the flock.

THE TRANSMISSION OF THE ORGANISMS THROUGH THE EGG

A large number of eggs laid by the ducks was examined bacteriologically. After collection, the eggs were incubated at 37° C. for 24 hours, removed from the incubator, dipped in methylated spirit and flamed. Each egg was then broken through a sterile funnel into 100 c.c. of peptone broth, to which was added brilliant green to a final concentration of about 1/75,000. The growth resulting after 24 hours' incubation was plated on MacConkey's medium. Alternatively, eggs were broken into peptone broth in a similar manner and after 24 hours' incubation the growth was streaked on 1/75,000 brilliant green agar plates. In all, 2,436 eggs were cultured, comprising 1,000 eggs laid while the ducks were isolated in the coops, 1,121 eggs laid in the breeding pens during the first and second breeding experiments, but not used for hatching, and 315 infertile eggs and dead-in-shell embryos from these same ducks. Neither of the organisms under study was recovered from any of these eggs.

BREEDING EXPERIMENTS

Two breeding pens of ducks were made up: Pen I contained reactors to *S. enteritidis* and Pen II reactors to *S. typhi-murium*. The titres of the ducks (Table III) at the commencement of the experiment are shown under the "third test." The trap nesting and hatching experiments were carried out on eggs laid during the three-monthly period between the third and fourth tests, and are recorded in detail in this table. After the fourth test the birds gradually ceased production until the seventh and eighth tests, when a further small breeding experiment was undertaken.

No trapnesting was attempted in the second experiment owing to shortage of labour, and the results are not recorded in Table III. Pen I consisted of eleven ducks, reactors to *S. enteritidis*, a number of which had been included in the first experiment (Nos. 244, 56, 247, 442, 608, 450 and 392). A total of 140 eggs laid between March and May was examined bacteriologically. Seventy-nine were incubated, 30 were infertile, 28 dead-in-shell, and 21 hatched. The second pen was mixed and contained reactors both to *S. typhi-murium* and *S. enteritidis*; 14 birds in all. Some of these had been in the first experiment (Nos. 646, 10, 360, 649 and 96). From these birds a total of 340 eggs was examined: 89 were incubated, 37 were infertile, 43 dead-in-shell and only nine ducklings hatched. The drakes used throughout both experiments were from a different source and had consistently negative blood tests.

Table III

Record of First Breeding Experiment

	Duck No.	Blood Test Records: Farm Test 1st Oct.	2nd Nov.	Laying: 3rd Mar.	Laying: 4th May	Non-laying: 5th Dec.	Non-laying: 6th Mar.	Laying: 7th Mar.	Laying: 8th May	Total No. of Eggs Laid*	No. of Eggs Cultured	No. of Eggs Set	No. Infertile	Dead in Shell	Ducklings Hatched	Condition of Ovary at Autopsy	Result of Bact. Findings
S. enteritidis	56	1/20	0	0	0	0	0	1/20	1/50	48	38	10	3	2	5	Inact. deg.	0
	181	1/50	1/1000	1/100	1/500	Died	—	—	—	2	2	—	—	—	—	Inact. nor.	+
	220	1/20	0	0	0	0	Died	—	—	47	41	6	0	3	3	" "	0
	244	1/50	1/200	1/100	1/500	1/20	1/50	1/50	1/100	55	45	10	4	4	2	Inact. deg.	0
	247	1/50	1/5000	1/50	1/50	0	1/20	1/50	1/20	51	42	9	5	4	0	Act. deg.	0
	392	1/50	1/20	0	0	0	0	0	0	33	27	6	1	2	3	Act. nor.	0
	442	1/20	1/1000	1/50	1/50	0	0	1/20	1/50	48	39	9	6	0	3	Inact. deg.	0
	450	1/50	0	1/50	1/20	0	0	0	0	53	43	10	3	2	5	Act. nor.	0
	608	1/20	1/20	1/20	1/20	0	0	0	0	29	25	4	3	0	1	Act. deg.	0
	770	1/50	1/100	1/100	1/50	Died	—	—	—	—	—	—	—	—	—	Inact. nor.	+
	659	1/50	1/50	1/50	1/50	1/20	Died	—	—	38	32	6	2	2	2	" "	0
							Total			404	334	70	27	19	24		
S. typhi-murium	10	1/50	1/200	1/100	1/100	1/20	1/20	1/50	1/20	9	6	3	1	2	0	Act. deg.	0
	49	1/50	1/20	1/20	1/20	1/50	1/50	Died	—	16	10	6	6	0	0	Inact. nor.	0
	139	1/20	0	0	Died	—	—	—	—	1	1	0	0	0	0	Act. deg.	0
	198	1/50	0	0	1/50	0	0	0	1/20	18	11	7	6	1	0	Act. nor.	0
	253	1/50	1/50	1/50	1/50	Died	—	—	—	65	44	21	17	1	3	Inact. deg.	0
	280	1/50	0	0	0	Died	—	—	—	71	45	26	10	12	4	" "	+
	360	1/50	1/20	1/50	1/50	0	1/20	1/50	1/20	72	49	23	4	9	10	Act. nor.	0
	96	1/50	1/200	1/100	1/100	1/50	1/20	1/50	0	49	31	18	16	1	1	Inact. deg.	0
	649	1/20	0	0	0	0	0	1/100	0	82	53	29	8	14	7	Act. nor.	0
	646	1/20	0	0	0	0	0	0	0	85	57	28	12	11	5	Inact. nor.	0
							Total			468	307	161	80	51	30		
							Total both pens			872	641	231	107	70	54		

* These numbers only refer to the eggs laid during the first breeding experiment carried out during the three-monthly period between the third and fourth blood tests.

Act. = Active
Deg. = Degenerate.
Inact. = Inactive.
Nor. = Normal.

The breeding results in both experiments were very disappointing, the number of ducklings hatched being very small. Conditions, however, were unfavourable, the birds being at least four years old, or older, and incubation conditions were poor. Nevertheless, no losses from any causes were experienced in the 84 ducklings hatched ; all were successfully reared without any special precautions, and when tested at three and five months old, no reactors were disclosed.

No definite conclusions can be drawn from these experiments. In the first breeding test, only two ducks from Pen I and one duck from Pen II, three out of five ducks which died before the conclusion of the test, were found on cultural examination to be definitely infected. These subsequently proved to be the only infected birds of those originally placed in the breeding pens, although four *S. enteritidis* reactors in Pen II in the second test were also found infected (Nos. 468, 338, 276 and 112). Fourteen out of the remaining 27 ducks showed pathological changes of the ovary at *post-mortem* examination.

Comparison of the egg-laying records shows that the reacting ducks laid equally as well as the non-reacting ducks, so that all facilities were present for transmission of infection through the egg. The results merely emphasise the difficulties associated with endeavours to demonstrate egg transmission by this method. One is led to the conclusion that the conditions under which the birds were kept at the laboratory were favourable for the gradual elimination of infection, and that no reinfection was occurring.

The failure of bacteriological examination to show infection in embryos and eggs, and the non-appearance of the disease in the ducklings, indicates that the number of infected eggs laid by infected ducks must be very small, so small as to be negligible. This evidence, slight as it is from the smallness of the experiment, does at least support the conclusions drawn from field observations.

DISCUSSION

The most obvious point arising from these investigations is that blood testing was not effective in reducing the percentage of reactors in the breeding flock. Van Oijen (1940), on the other hand, found that measures based on the agglutination test were successful in reducing the percentage of reactors in a flock from 23 to nine, of infected eggs from 14·3 to nil, and of ducks laying infected eggs, from 30 to nil. Although on the farm the non-removal of reactors and the inefficiency of the hygienic measures may have been to blame, the periodic testing at the laboratory showed that fluctuations in titre can result in as many as 17 per cent. of infected birds failing to react at any one test. Similarly, both Zwannenberg (1937) and Wanner (1937) found the agglutination test to be unreliable in the detection of carriers in a heavily infected flock. There also appeared to be some correlation between serum titre and egg production. With the decrease in egg production the titre of reacting birds fell,

and some birds shown later to be infected, gave negative serum reactions. This is in agreement with the findings of Warrack and Dalling (1932). Conversely, there was some increase in titre when birds returned to laying ; ducks which had become negative returned a positive reaction at later tests. To obtain maximum efficiency in diagnosis blood testing should, therefore, be carried out during the laying period and repeated testing at intervals during this period seems necessary. Since there was a striking increase in the percentage of reactors in the third- and fourth-year birds (3 per cent. in the first- and second-year birds and 13·5 per cent. in the third- and fourth-year birds) presumably due to ground infection, the retention of birds over two years of age, should be discouraged ; their elimination would greatly assist in the reduction in the number of carriers.

Jansen and Van Haselen (1939), in periodic testing on 100 ducklings, kept on ground known to be infected with *S. enteritidis*, found that no reactors were observed until the ducklings reached maturity, although during this time eleven birds died of the disease. Our field observations on the routine testing of infected flocks in this country support their findings, so that a further proviso to routine testing is that the birds should have reached maturity, with a high percentage in production, and that further testing is necessary as younger birds come into lay.

Since 67·5 per cent. of birds with a titre of 1/100 or over showed infection on cultural examination, this titre undoubtedly indicates active infection. In birds with a lower titre the organisms were recovered with much less frequency (38·5 per cent.), but cognisance must also be taken of the fact that the organisms were isolated from six birds whose serum titre was never higher than 1/20. Bearing in mind the low economic value of the individual duck, it would be essential in the routine blood testing of an infected breeding flock to regard reaction at a dilution of 1/20 as the criterion of infection.

Zwannenberg (1937) also found correlation between serum titre and degeneration of the ovary and he did not observe normal ovaries in any bird showing a higher titre than 1 in 100. He also found that the birds showing the highest titres laid the greatest number of infected eggs.

The most striking feature of the cultural work was the frequency with which the organisms were recovered from the ovary. Since this lends support to the findings of previous workers, Scott (1932), Warrack and Dalling (1932, 1933), Jansen (1936), Wanner (1937), and Zwannenberg (1937) and to the egg transmission theory, an attempt was made to correlate these findings with the activity of the ovary and the laying of infected eggs. It was found, however, that out of the 45 infected ovaries only five were active and consequently potential sources of transmission at the time of examination. Although the remaining birds from which the organisms were recovered from the ovary must be considered as potential transmitters when and if the ovary was functioning, this was not borne

out by our negative breeding experiments or by the negative results obtained on the cultivation of over 2,000 eggs.

This is the agreement with the findings of Hole (1932). Even those workers who have succeeded in isolating the organisms from eggs have found eggs to be infected in only an extremely small percentage. At the same time we have been unable to find any references to positive breeding experiments. Furthermore, the discrepancy between the infection in the ducklings, which was established as mainly due to *S. typhi-murium* (Garside and Gordon, 1940) and the blood testing results of the breeding flocks, which disclosed four times as many reactors to *S. enteritidis* as to *S. typhi-murium*, cannot fail to have been noticed. This would warrant the surmise that *S. enteritidis* was not transmitted in the egg, and this is supported by the observation noted in our previous report that the persistence of carriers in the breeding flock did not influence the course of the disease in the ducklings. Further, attention must be drawn to the fact that in this *S. typhi-murium* infection in the ducklings the peak mortality occurred as late as the 12th to 14th day after hatching, and that the mortality rate was reduced from 30 per cent. to 3 per cent. by improved hygiene on the rearing farm, while concurrently no losses occurred in ducklings bred from the same stock and reared in batteries, again indicating that in this outbreak at least the cycle of infection was not *via* the egg.

In connection also with egg transmission, it is of interest to note that in the overwhelming majority of reported cases of food poisoning of human beings in which ducks' eggs are suspect, the recovered organism is *S. typhi-murium* and not *S. enteritidis*.

Although they are not altogether conclusive, all our experimental findings bear out our previous contention which was based on field observations (Garside and Gordon, 1940) that in Salmonellosis in ducks, egg transmission, if it does occur, plays a minor role in the dissemination of infection. It is also evident that in the control of this outbreak the practical value of the routine blood testing of breeding stock was open to question, and although this is supported by other workers it does not agree with results obtained in the Netherlands where it is claimed that mortality in ducklings has been lowered by breeding only from non-reactors. Whether routine testing of duck flocks is justified in view of the known pathogenicity of infected eggs to human beings is another matter, but if it is, it would seem that testing for the presence of *S. typhi-murium* is all that is required.

SUMMARY

The failure to eliminate reactors to *S. typhi-murium* and *S. enteritidis* by routine blood testing from a large flock of breeding ducks is recorded. Reasons for this failure are discussed, and include the persistent reinfection of adult ducks, together with fluctuation in the serum titre of reacting ducks associated with ovarian activity.

The recovery of *S. enteritidis* var. Essen is recorded.

It was impossible to demonstrate the transmission of the organisms through eggs from these reacting ducks, either by cultural examination of over 2,400 eggs, or in ducklings hatched from their eggs.

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NICOTINIC ACID DEFICIENCY IN THE PIG AND GUINEA-PIG

I.—THE EFFECTS OF A "PELLAGRA-PRODUCING" DIET UPON THESE SPECIES

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INTRODUCTION

Nicotinic acid, β-pyridine carboxylic acid, is one of the water-soluble, heat-stable compounds included in the "vitamin B_2 complex." Human pellagra is essentially a nicotinic acid deficiency, and the majority of patients respond to nicotinic acid, or nicotinamide, although in some cases other deficiencies may be present. The monkey, dog ("canine blacktongue"), fox, and pig require nicotinic acid in their diets, while there is evidence that the cat, guinea-pig, pigeon, chick and many other higher species may need it. The rat, which is commonly employed in nutritional studies, appears to be independent of a dietary source of nicotinic acid: this fact was not only responsible for delay in the identification of nicotinic acid as the "pellagra-preventive" or "anti-blacktongue" factor, but has also made it necessary to look for another laboratory mammal for purposes of biological assay.

In the case of the pig, the first published description of nicotinic acid deficiency (Birch, Chick, Martin and Martin, 1937; Chick, Macrae, Martin and Martin, 1938a, 1938b) was of great interest to veterinarians, since the condition produced bore a close pathological similarity to naturally occurring necrotic enteritis. Many subsequent publications have suggested that nicotinic acid deficiency is an aetiological factor in field cases of necrotic enteritis, and, in the U.S.A., proprietary preparations containing nicotinic acid are on sale as specific therapeutic agents for this condition.

Paratyphoid or necrotic enteritis is under investigation at this laboratory, and in June, 1940, a study of the relationship between nutritive deficiency and the disease was begun. At the same time the possibility of using the guinea-pig as a test animal for a nicotinic acid deficient diet was investigated. The present communication provides (1) a brief review of the literature upon nicotinic acid deficiency in the pig and guinea-pig, and (2) an account of some preliminary experiments in which "pellagra-producing" diets were fed to these species. Experiments concerning the specific effects of nicotinic acid deficiency upon paratyphoid infection in the pig will be dealt with in a subsequent paper from this laboratory.

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REVIEW OF THE RELEVANT LITERATURE

Chick and her co-workers (Birch *et al.*, 1937 ; Chick *et al.*, 1938a, 1938b) fed Large White weaners on a pellagra-producing diet of the Goldberger or high-maize type. They pointed out that the pig could not be reared on maize only and quoted Dunlop (1935) and Miller and Rhoads (1935) in evidence of the fact that protein and mineral deficiency alone were not responsible for this failure. In five to ten weeks a severe diarrhoea and illness developed, and an outbreak of pig paratyphoid was suspected but could not be confirmed bacteriologically despite close pathological resemblance. Mr. M. O. J. McCarthy, M.R.C.V.S., who carried out the bacteriological examinations, kindly informs us that the only suspected organism, obtained in single isolation from one animal was not *S. suipestifer*. Control pigs receiving 4 or 8 per cent. of yeast as supplement remained healthy, and affected animals responded dramatically to nicotinic acid therapy. Deficiency symptoms did not develop in markedly coprophagous animals or in those bedded on wheaten straw, while the replacement of the maize by whole wheat or whole barley rendered the unsupplemented basal diet just satisfactory for control pigs. Dried whey was effective in preventing symptoms. For a précis of the clinical and pathological findings in this and other vitamin B_2 complex deficiencies, as recorded by the Cambridge workers—see Worden (1939). McEwen (1937) recorded necrotic enteritis in a herd in which swine fever virus, specific bacterial infection, helminthiasis, coccidiosis or sanitary factors could not be blamed, and quoted experimental work (at Wye) which indicated a nutritional factor in necrotic enteritis.

Similar field observations have been made from this laboratory, but the diets concerned would not appear to have been deficient in nicotinic acid. Hughes (1938) produced " pellagra " in young Duroc Jersey and Poland China pigs, and prevented the condition by treatment with vitamin B_1, riboflavin, nicotinic acid and filtrate factor. Hughes (1943) has since shown that 10 mg., but not 5 mg., of nicotinic acid per 100 lb. body weight daily will prevent deficiency symptoms when other known factors are fed, and has recorded failure to produce pig pellagra when the pen floors harboured yeasts and moulds, which apparently contained nicotinic acid. Hughes did not observe a dermatitis, as the Cambridge workers had done.

In Australia, McLennan, MacIndoe and McKenna (1938) reported an enteritis syndrome resembling that described by the Cambridge workers, and in the U.S.A. Madison, Miller and Keith (1939) recorded a similar condition which responded rapidly to daily doses of 50 mg. of nicotinic acid. Fairbanks and Curzon (1940), Hastings (1940) and Hawthorne (1941) likewise reported success in the treatment of necrotic enteritis with nicotinic acid, while Hawthorne, but not Hastings, found similar treatment effective against " swine dysentery." This last observation is not supported by Edgington (personal communication). These clinical observations made no attempt to establish whether or not the necrotic enteritis was associated with *S. suipestifer* infection, but at Michigan Davis and his colleagues (Davis, Freeman and Madson, 1940 ; Davis and Freeman, 1940) claimed that nicotinic acid did, in fact, protect against this infection, a finding which was not corroborated by Edgington, Robinson, Burroughs and Bethke (1942), and which will be discussed in a later paper from this laboratory.

More complex vitamin B_2 deficiencies have been produced in the pig, *e.g.*, by Blissett and Golding (1931), Miller and Rhoads (1935), Chernyshev (1939), and by Wintrobe and his colleagues (Wintrobe, Mitchell and Kolb,

1938; Wintrobe, Miller and Lisco, 1940, etc.), and in some of these there seems little doubt that nicotinic acid was among the missing factors.

In the case of the guinea-pig, the only relevant communication at the time of our studies was that of Harris (1939), who stated that animals restricted to a modified cooked Goldberger diet, supplemented by vitamins A, B_1, C, D, E and riboflavin, rapidly lost weight, exhibited salivation, and succumbed. "The addition of nicotinic acid to the diet permitted some growth and lowered the mortality rate," although better results were obtained by the addition of 15 per cent. dried yeast.

EXPERIMENTAL

In an attempt to reproduce the deficiency syndrome described by Birch *et al.* (1937) and by Chick *et al.* (1938a, 1938b), younger animals were used, *i.e.*, pigs nearer the age when necrotic enteritis is seen. The young pigs were housed with their mothers and given the different diets as "creep-rations" so that from the day of weaning they received the experimental rations only. This did not allow of animals from different litters being grouped together unless abnormally early weaning was to be adopted. There seemed to be no other simple method of determining the effects of a deficiency diet on very young pigs.

Three litters of Wessex Saddlebacks were available. The growth rates even of the normal controls were very disappointing, bearing in mind the excellent performances previously obtained with pigs bred at this laboratory. Unfortunately, changes in the strain of pig and in the management had had to be made shortly before these experiments commenced, and these may have been largely responsible for the poor results. Two of the sows, Nos. 649 (supplemented group) and 650 (basal group) were afterwards disposed of because of the poor quality of their subsequent litters. Sow No. 651 (stock ration group) was retained but only reared good litters when allowed free range.

The effect of a pellagra-producing diet was tried also on guinea-pigs, with the additional purpose of finding out whether or not these animals could be used for testing pig-rations.

Pig Experiment

The three litters, farrowed out-of-doors, consisted of seven, eight and four young pigs, respectively. The litter of seven (Group I) was moved indoors with its sow (sow 651) at four weeks of age, creep-fed with a standard ration (Table I), weaned at eight weeks, and afterwards kept as a normal control group.

[One animal (No. 915) which scoured was destroyed a fortnight after weaning as a "bad doer." No abnormalities were detected on *post-mortem* examination except a slight catarrh of the large intestine. Cultures from the spleen, liver, kidneys and a mesenteric lymph node were negative.]

The second litter, of eight (Group II) fed with a "pellagra-producing" diet very similar to that given by the Cambridge

workers, may be termed the " basal group " (Table I). The mineral mixture, devised with the help of Dr. H. H. Green, was modified by the inclusion of trace elements. This litter was housed at 13 days of age, and the basal diet was provided as a creep-ration. It was weaned at eight weeks of age. The sows were fed on our stock ration, care being taken that the piglets did not have access to it.

The litter of four only (Group III) formed the " 4 per cent. yeast controls," or supplemented group. This also was housed at 13 days of age, and fed and kept in the same way as the basal ration group except that the diet contained 4 per cent. added brewers' yeast.

All pigs were weighed weekly and bled at intervals for serological and for limited haemoglobin tests. The sera remained negative throughout when tested against both " O " and " H " antigens of *S. suipestifer*. In addition, samples of faeces were collected

TABLE I

Stock Ration	*Parts*	Basal Ration	*Parts*
Weatings	336	Whole white maize (ground) ...	80
Barley meal	224	Pea meal	10
Fish meal	56	Casein (Glaxo extracted ashless)	4·5
Flaked maize	76	Cod-liver oil	3
Common salt	2·5	*Mineral mixture	2·5
Cod-liver oil	7		

* *Mineral Mixture*

	Parts		*Parts*
Calcium carbonate (limestone)	40	Ferric citrate	2
Calcium phosphate (bone ash)	36	Copper sulphate	0·3
Sodium chloride (common salt)	15	Cobalt sulphate	0·1
Potassium chloride	2	Manganese sulphate	0·3
Magnesium carbonate	3	Potassium iodate	0·1
Ferric oxide	2		

daily, and from them cultures were made on brilliant green-neutral red-lactose agar (Slavin, 1943) and on other media. Neither *S. suipestifer* nor any other pig pathogen was isolated at any time. Samples of faeces from the three sows and from the piglets were examined by Dr. E. L. Taylor. Although a few coccidia, *Balantidium coli*, and ova of *Oesophagostomum dentatum* were found in the maternal samples, those from the piglets were negative. Strict care was taken to exclude infection and to prevent the animals eating materials other than their diets: hence, the sows' faeces were removed frequently. Wooden boards were used as bedding, and access was provided to open air on concrete runs only. The sties were of brick and concrete. Many of the pigs maintained a high fibre content in the faeces by eating the wood; wood is, however, not a known source of nicotinic acid. The boards had already been used many

times for pigs and had probably soaked up faeces and disinfectant.

The experiment was continued with all three groups until eleven weeks after weaning. The normal controls were then withdrawn for use in other experiments. The pigs which survived on the basal diet and those in the supplemented group were retained on experiment for seven weeks longer before they were placed on a stock ration. The weights are shown for three weeks on this stock ration.

The pigs of all groups fed well (except as noted below) during the whole of the experiment. The growth of the stock group was poor compared with that expected of good quality pigs on such a ration, but was about equal to that of the supplemented ration group and much better than that of the basal group. While neither the stock ration group nor the supplemented group did well, there was no evidence of ill-health during the first two weeks (other than in pig 915 noted above) apart from a scouring, which then disappeared spontaneously. The average weight-gains of the groups are shown in the graph and the actual weights for each pig in Table II. The three groups ran roughly parallel until three weeks after the weaning of those fed the basal and supplemented rations, when the basal began to drop behind the supplemented group. The supplemented continued to grow at about the same rate as the stock ration group but the growth was irregular. It was expected that the basal would continue to drop behind the supplemented group but the former rose sharply during the eleventh week after weaning, following the death of pigs 920 and 921. The growth of these two pigs was not more irregular than that of the other pigs in this group and their absence does not explain the sudden rise in growth rate. The growth rate was thenceforward about doubled and the curve in the graph runs about parallel to that of the supplemented group. Spontaneous recovery seemed to take place.

The basal group pigs were backward and small, but quite lively and the fine condition of their skin was striking.

The haemoglobin values (see Table III) of the supplemented controls (Group III) were on the low side of normality but the normal stock ration controls (Group I) were well up to standard. The basal group pigs (Group II), on the other hand, were mostly anaemic, particularly Nos. 920 and 921, which had very low values. As in the other two groups, there was an initial scouring which cleared up within a fortnight, but there were indications of inappetence and seven weeks after weaning a blood-stained diarrhoea was found in the pen. This persisted for about a week, at the end of which two of the pigs (Nos. 920 and 921) were noted to be very ill and not feeding. Both died almost exactly nine weeks after weaning. The *post-mortem* findings were as follows :—

Pig 920.—Numerous greenish yellow diphtheritic deposits on the mucous membrane throughout the whole length of the caecum and colon. Deposits thin and easily detached, of irregular outline and up to about 1 inch greatest

TABLE II

WEIGHT OF PIGS IN LB.

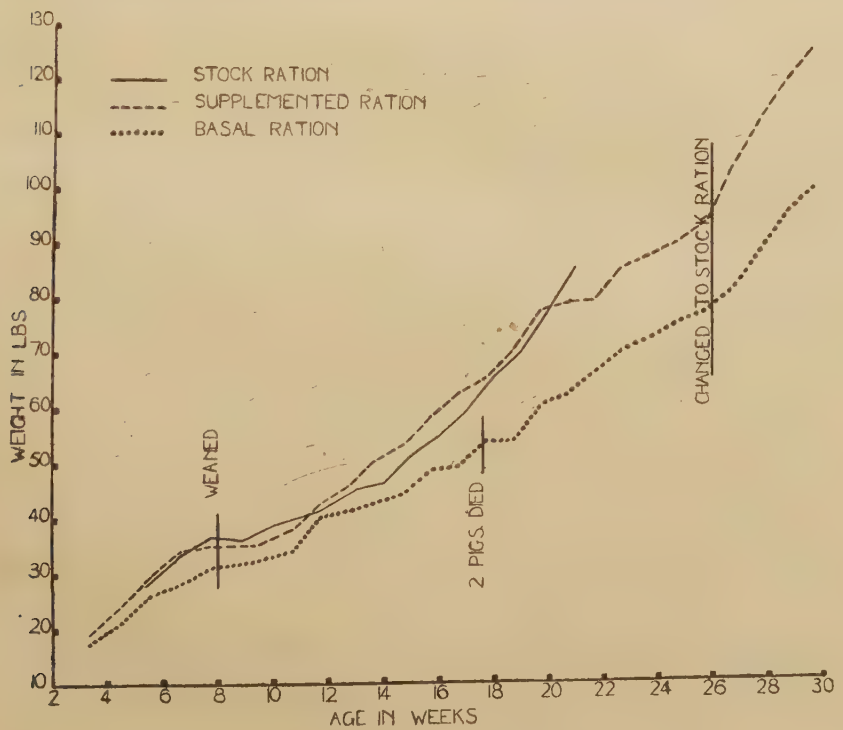
Group	Pig No.	Age in Days																										
		23	31	39	47	55	66	76	83	90	97	104	111	118	125	132	139	146	153	160	167	174	181	189	195	202	209
Stock Ration	912			30	37·5	45	45·5	46	48·5	54·5	57·5	64·5	67	75	82	89	96	105									
	913			32	37·5	42	42·5	42·5	47·5	52·5	52	58	60	67	71	77	86	100									
	914			25	28	33	32	30	33	34·5	32·5	36·5	42	46	49	54	61	62									
	916			27	32	34	28	33	35	36	40	45	49·5	54	59	61	68	81									
	917			25	29	35	36	37·5	41	45	44	47·5	53	54	62	67	74	81									
	918			30	36	40	42	43	44	48·5	48	55	58	61	66	70	76	82									
Basal Ration	919	18	24·5	29·5	33	36	37	38	42·5	47	50	51	57	62	66	70	79	82	90	98	102	105	107	114	120	130	130
	920	17·5	19·5	24	27	30	29·5	32	35	35·5	35	36	41	36	+												
	921	18·5	20	26	28	27·5	30	29	33	35	37	38	43	43	+												
	922	18	21·5	28	28	34·5	33	36·5	42	40·5	42·5	45	46	47	43	43	47	47	49	50	50	51	56	58	62	67	75
	923	18	21	27	27·5	32	32·5	33	42	42·5	41·5	45	46	46	48	47·5	56	57	57	54	60	60	60	68	73	80	92
	924	17	22·5	27·5	29	32·5	36	36·5	44	43·5	45	45	54	54	54	52·5	57	65	65	67	72	76	73	78	87	95	97
	925	16	18·5	23	26	30	31	34	42·5	43	47	47·5	52	55	57	57·5	63	65	70	77	76	83	87	90	97	103	106
	926	17	22	25·5	27	28	33	34·5	40	41	43·5	45	49	51	53	52	56	57	66	67	72	77	80	81	87	95	97
Supplemented Ration	927	21·5	27	34	40	41·5	44·5	49	55·5	60	67	74	81	87	89	98	107	107	107	117	120	117	122	132	141	150	154
	928	17·5	22·5	29	34·5	35·5	34	38	43	47	50	52·5	58	58	62	67·5	72	74	76	82	81	87	91	101	110	117	127
	929	18	25	30	33·5	35	35	36	37	40·5	43	42·5	47	53	51	56·5	62	66	65	68	74	73	79	91	95	105	110
	930	19	23	25	28·5	27·5	27	30·5	33	35	39·5	43·5	46	51	57	60	67	67	67	73	73	77	81	92	101	105	111

diameter, the largest deposits being in the region of the ileo-caecal valve. Numerous bright red areas and petechial haemorrhages in the mucous membrane. Mucous membrane of the fundus of the stomach slightly reddened. Abdominal lymph nodes haemorrhagic. Lungs congested.

TABLE III
HAEMOGLOBIN IN SAMPLES TAKEN AT FIVE WEEKS AFTER BEGINNING OF EXPERIMENT

Group	*Pig. No.*	*Hb. in Grammes per cent.*	*Group*	*Pig. No.*	*Hb. in Grammes per cent.*
Stock ration	912	12·1	Basal ration	919	10·2
	913	12·1		920	6·5
	914	11·1		921	3·9
	916	11·6		922	9·2
	917	12·4		923	9·3
	918	12·8		924	11·8
				925	7·9
	927	9·0		926	7·9
Supplemented ration	928	8·4			
	929	9·0			
	930	10·0			

Excess fluid in the pericardial sac. Soft palate inflamed. Submaxillary lymph nodes haemorrhagic. No *Salmonella* organisms were recovered from the liver, spleen, kidneys, lungs, mesenteric nodes or from bowel scrapings.



Pig 921.—Excessive serous fluid in the abdominal cavity. The mucous membrane of the whole large intestine, with the exception of about the last foot, deep red, the reddening being most marked along the tops of the mucous membrane folds. A large part of the mucous membrane of the stomach pale, wrinkled and covered with tough mucus. Lymph nodes of abdominal cavity haemorrhagic. Sero-fibrinous pleurisy, with large amount of serous fluid. Pericardial sac dilated with clear serous fluid. Lungs dark but not hepatised. Interlobular septa of lungs dilated with gelatinous fluid. *S. suipestifer* was recovered from the mesenteric lymph nodes but not from any other organ. It is extraordinary, in view of the low state of the pig for some days before death, that the organism was confined to the mesenteric glands. It is concluded that the pig was a carrier of *S. suipestifer* and that the organism had no connection with any disease process.

GUINEA-PIG EXPERIMENT

Few guinea-pigs were available, but 12 newly-weaned ones, five weeks old, were selected from four litters and divided into four groups of three. The four groups were provided with diets as follows :—

Group A : Normal stock diet of oats, bran and greenfood.

,, B : Pellagra-producing diet identical with that fed to the pigs, but supplemented by 5 mg. ascorbic acid per animal per day.

,, C : Pellagra-producing diet + ascorbic acid + 2 mg. nicotinic acid per animal per day.

,, D : Pellagra-producing diet with 15 per cent. added yeast + ascorbic acid.

The cod-liver oil, ascorbic acid and nicotinic acid were administered by individual dosing. (The ascorbic acid was kindly supplied by Messrs. Roche Products, Ltd., Welwyn Garden City, Herts). The animals were maintained in metal cages with wooden boards for flooring, and provided with water. No attempt was made to prevent coprophagy, except that the cages were cleaned out daily, but in view of the oft-noted ability of this species to take faecal pellets from the rectum* it is doubtful if any procedure short of collaring could inhibit the practice. Group A, the normal controls, grew normally over a period of nine weeks, by which time all the animals in Group B (basal diet only) and Group C (basal diet + nicotinic acid) had died and there had been one death in Group D (15 per cent. yeast controls). Groups B, C and D all grew badly with the exception of a single animal in the nicotinic acid group which, however, died after seven weeks. Administration of nicotinic acid by mouth (up to 10 mg. daily) to two members of Group B did not appear to exercise any effect on the course of the disease.

* While there is no definite evidence that the guinea-pig regularly practises coprophagy of the type normally exhibited by the rabbit, *i.e.*, " pseudo-rumination," it would be of considerable interest to investigate this point in some detail. Suggestions that a " less regular " rhythm than that of the rabbit does occur in this species was made by H. Winterstein (1911).

It is clear from these limited experiments that a pellagra-producing diet does not support health or life in the guinea-pig, but in view of the failure of nicotinic acid to protect against or to cure the condition, and of the poor performance of the animals in Group D, it is probable either that another deficiency, or that the physical unsuitability (*i.e.*, unsuitability to the digestive tract of this species) of the diet, was concerned. Studies involving larger numbers of animals might have indicated that nicotinic acid exerted some protective action as Harris (private communication) has pointed out to be the case in other laboratories. The vitamin requirements of the guinea-pig are now receiving attention. (See, *e.g.*, Sober *et al.*, 1942.)

One of the groups receiving the unsupplemented basal diet was found to have a friable liver, but otherwise there did not appear to be any significant pathological or cultural findings other than the poor coat condition in all but the normal control animals. Diarrhoea was noted in the basal group, and pasty faeces appeared later in both the nicotinic acid and the yeast groups. One of the basal group which survived for nine weeks presented a peculiar " rat-like " appearance during the later stages, and exhibited also some loss of hair on the abdomen, and sores on the underside of the feet. The last changes were in all probability due to the absence of proper bedding. In some instances death was preceded by convulsions.

DISCUSSION

The experiments reported in this paper provide further evidence that a condition clinically and pathologically resembling necrotic enteritis, as seen in pig paratyphoid, can be induced by feeding pigs on a pellagra-producing diet. The results, however, have been less striking than those originally reported at Cambridge by Chick and her co-workers, six out of eight animals in the deficient group surviving until the end of the experiment, when they were 25 weeks of age. This may have been due to unascertained causes which prevented full depletion of most of the animals or which enabled them to recover spontaneously. Spontaneous recoveries from a severe macrocytic anaemia produced by a prolonged partial vitamin B_2-complex deficiency were reported in pigs 28 to 35 weeks of age by Smith, Reiser and Harrell (1941). The animals in our experiments may have been assisted in recovery by nicotinic acid-synthesising moulds and yeasts on the pen-floors (see Hughes, 1943), by nicotinic acid in the pea-meal (this possibility was eliminated in later experiments by the use of synthetic diets) or by the initial poor quality of the basal group which grew so slowly that its nicotinic acid requirements were consequently subnormal. It is well recognised in certain other deficiency diseases, *e.g.*, rickets in the human baby, that a slower rate of growth may result in a less severe deficiency manifestation.

The excellent skin condition of all animals in the experiment is noteworthy. Taken in conjunction with Hughes's (1943) findings, the opinion is hazarded by us that in the experiments of other workers parasitism may have been a complicating factor, for a hitherto latent infestation with mange is commonly known suddenly to become clinically apparent when the animals fall into bad condition.

A nicotinic acid deficiency, and even a frank pellagra, may occur naturally in pigs in the maize-growing areas of the world. There is as yet, however, no satisfactory published account of its occurrence in Great Britain, and despite the relatively high requirements of the growing pig for the vitamin, the deficiency is probably rare in this country. It is remarkable that all workers who have carried out similar experiments have found some of their animals capable of overcoming the deficiency.

The limited experiments on guinea-pigs, while confirming the fact that a pellagra-producing diet does not support health or continued life in this species, do not indicate the usefulness at present of this animal as a test subject for nicotinic acid.

SUMMARY

The literature of nicotinic acid deficiency in the pig and guinea-pig has been reviewed.

It has been confirmed that a necrotic enteritis may occur in the young pig which is provided with a " pellagra-producing " diet, and which is not infected with *Salmonella suipestifer*.

A " pellagra-producing " diet failed to support life or health in the young guinea-pig, and proved less suitable than a normal stock diet even when supplemented by 15 per cent. of brewer's yeast.

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YEAST AS A PROTEIN SUPPLEMENT FOR PIGS; FURTHER OBSERVATIONS ON ITS RACHITOGENIC EFFECT

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INTRODUCTION

In a previous paper (Braude, Kon & White, 1943) rickets was recorded in pigs placed on diets in which brewer's yeast took the place of fish or meat meal as protein supplement. The rickets could be prevented by including small amounts of cod-liver oil in the diet. The present paper describes experiments confirming the rachitogenic action of high yeast diets and some attempts to elucidate this effect.

EXPERIMENT 4*

A pilot experiment was undertaken to determine whether optimal bone formation could be obtained with a good normal fattening diet or whether addition of cod-liver oil to it was necessary, and also whether the vitamin D in cod-liver oil not only prevents but also cures the symptoms evoked by high yeast diets.

A litter of eight Large White pigs, twelve weeks old, was used. Three were given a diet in which the protein supplement was white fish meal; three received the same diet to which was added cod-liver oil ("controlled mixture") at a daily rate of 14 g. per pig, and the remaining two received 20 per cent. brewer's yeast instead of fish meal. The last two animals were the smallest in the litter but were otherwise normal. The experimental procedure was exactly similar to that previously used; the pigs were fed individually and the cod-liver oil was added daily. The experiment began on March 12th, 1942, and lasted 21 weeks. The fish meal diet contained weatings 61, crushed oats 15, flaked maize 10, dried grass 2, fish meal 12, all per cent. The yeast diet contained yeast 20, in place of fish meal, weatings 51, limestone 1·5, and salt 0·5, all per cent.; the other constituents were as in the fish meal diet.

RESULTS

As shown in Table I and Fig. 1, the pigs receiving the fish meal diet grew satisfactorily and showed no symptoms of rickets; those receiving cod-liver oil grew slightly better. The two pigs receiving 20 per cent. yeast showed typical symptoms of rickets already

* Experiments 1 to 3 are described in the previous paper.

described. (Braude *et al.*, 1943.) Food was first refused after four weeks and at the end of twelve weeks both animals were unable to rise and were considered unlikely to survive until the end of the experiment. The animals were now given daily doses of 40 g. of the " controlled cod-liver oil mixture " and were exposed to direct

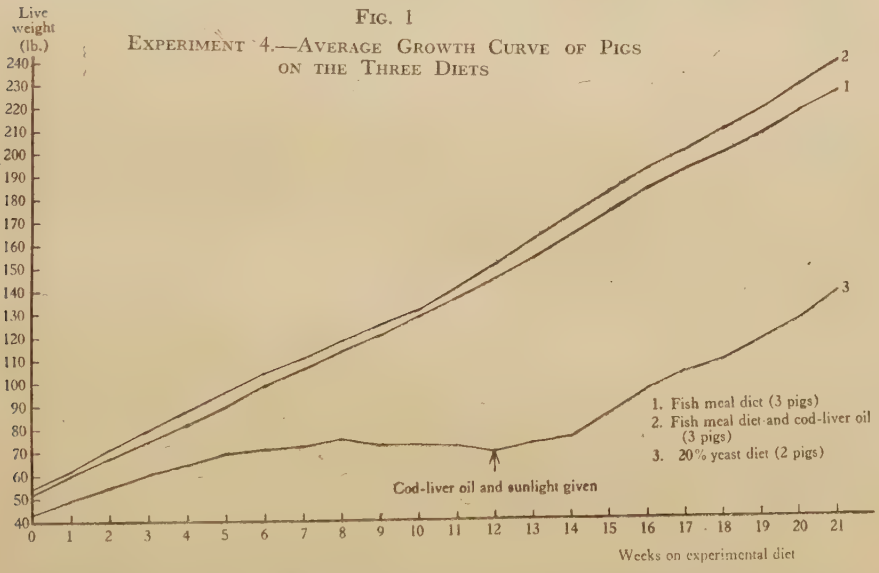
TABLE I

EXPERIMENT 4.—GROWTH OF PIGS AND FOOD CONSUMPTION.

	Diet.		
	Fish Meal (3 *Pigs*) *lb.*	*Fish Meal* + *C.L.O.* (3 *Pigs*) *lb.*	20 *per cent. Yeast* (2 *Pigs*) *lb.*
Initial live weight	52·6	54·3	44·0
Weight after 12 weeks ...	146·0	152·0	69·5
Average daily gain (12 weeks)	1·2	1·1	0·3
Final weight after 21 weeks ...	229·7	242·3	138·5
Average daily gain during last 9 weeks	1·3	1·4	1·1 (anti-rachitic treatment)
Pounds of meal per lb. live weight gain	4·1	4·0	—

FIG. 1

EXPERIMENT 4.—AVERAGE GROWTH CURVE OF PIGS ON THE THREE DIETS



sunlight for about one hour daily. In a few days the pigs began to eat, their appetite then rapidly improving, and after twelve days all food offered was consumed. Although for the first few days the animals had to be carried to the outdoor pen for sunlight treatment, they were soon able to walk and eventually lost all stiffness. Radiographs of the carpus were taken before treatment commenced and again five weeks later.

All the pigs were killed at the end of 21 weeks' experiment and the bones examined as already described. The ash contents of the bones of pigs receiving the fish meal diet were 51·5, 50·7 and 48·6 per cent., of those receiving cod-liver oil in addition, 52·4, 52·4 and 53·3 per cent., and of the two pigs on the 20 per cent. yeast diet, 52·9 and 52·2 per cent. It appears that addition of cod-liver oil to the fish meal diet produced a rise in the ash content; the high value of the ash content in the pigs receiving yeast is probably due to the nine weeks' treatment with cod-liver oil and sunlight.

Pathological Examination. No difference (Table II) was detected between the bones of pigs receiving the fish meal diet alone and supplemented with cod-liver oil. The two pigs on the 20 per cent. yeast diet showed very pronounced rickets after five weeks, and after twelve weeks the radiographic assessment was 10 (maximum).

TABLE II

EXPERIMENT 4.—CONDITION OF BONES AT END OF EXPERIMENT

Pig No.	*Diet.*	*Assessment.**		
		X-ray.	*Macroscopic Findings.*	*Histological Findings.*
2983	Fish meal	2	2	1
2986	„	1	2	1
2990	„	1	1	1
2985	„ + C.L.O. ...	2	1	1
2987	„ „ ...	3	1	1
2989	„ „ ...	2	2	1
2984	20 per cent. yeast ...	3†	4	3
2988	„ „ „ ...	4†	3	4

* The method of assessment was to indicate, as in the previous experiment, the condition of the bones by the numerals 1 to 10, representing progressively the least (1) to the worst (10) rickets.

†After 12 weeks on the yeast diet X-rays of both pigs gave an assessment of 10. By the end of the experiment both showed healed rickets and were assessed at 3 and 4 respectively.

Following five weeks' treatment with cod-liver oil and sunlight the animals became clinically almost normal and the radiographic assessment went down to 3. Macroscopically, the bones of the two pigs on the yeast diet showed healed advanced rickets, with grooves and pits on the articular surfaces of the elbow joints and wide and irregular epiphyseal cartilages.

This experiment thus confirms the rachitic nature of the symptoms and lesions and illustrates their rapid alleviation by cod-liver oil and sunlight. The addition of cod-liver oil to a good normal fattening diet did not significantly affect the structure of the bones, although there was a slight rise in the ash content.

EXPERIMENT 5

The rachitogenic action of high yeast diets may have been due, as suggested in our previous paper, to their high content of available

phosphorus. An attempt was now made to simulate this yeast effect by adding inorganic phosphorus, greatly in excess of that in a 20 per cent. yeast diet, to a diet not containing yeast (diet 2, Table III). For comparison, a normal diet was also fed, with meat and bone meal as protein supplement and Ca and P adjusted to the same levels as in the 20 per cent. yeast diet (diet 1). Further, a large excess of calcium was added to the 20 per cent. yeast diet to see whether it would inhibit the yeast effect (diet 4). Finally, calciferol was added to the 20 per cent. yeast diet to find out whether vitamin D was in fact the protective and curative agent of cod-liver

TABLE III

EXPERIMENT 5.—COMPOSITION OF DIETS

	Diet.			
	1	2	3, 5* & 6†	4
Wheat feed	48½	48½	36	36
Flaked maize	20	20	20	20
Ground oats	10	10	10	10
Potato meal	10	10	10	10
Dried grass	2	2	2	2
Meat and bone meal	9	9	—	—
Dried yeast	—	—	20	20
Calcium carbonate	—	—	1½	4
Salt	½	½	½	½
Sodium phosphate ($Na_2HPO_4.12H_2O$)...	—	6	—	—
Chemical Composition—				
Moisture	12·9	15·6	11·7	11·4
Protein	16·5	15·6	18·8	18·3
Oil	3·2	3·0	2·4	2·3
Carbohydrate	54·0	50·9	54·8	53·5
Fibre	6·5	6·1	5·3	5·2
Ash	6·6	8·5	7·0	9·3
Ca	0·9	0·9	0·9	2·1
P	1·0	1·54	1·0	1·0

* Diet 5 is as diet 3 but with the addition of 14 g. per pig daily of cod-liver oil (" controlled mixture "), the vitamin D potency of which was stated to be not less than 85 i.u./g.

† Diet 6 is as diet 3 but with the addition of 14 g. per pig daily of a solution of calciferol in arachis oil containing 85 i.u./g. of vitamin D.

oil (diet 6). The 20 per cent. yeast diet with and without cod-liver oil served as control diets (diets 5 and 3). The methods were exactly similar to those already used. Six different diets were thus given and each was replicated six times, the six pigs in each block being litter mates. The composition of the diets is given in Table III. The diets were fed for 22 weeks, the first block beginning in May and the last in July, 1942.

RESULTS

Growth and Symptoms of Rickets. (See Table IV and Fig. 2). The animals on diets 5 and 6, *i.e.*, the 20 per cent. yeast diets supplemented with cod-liver oil or calciferol, respectively, grew satisfac-

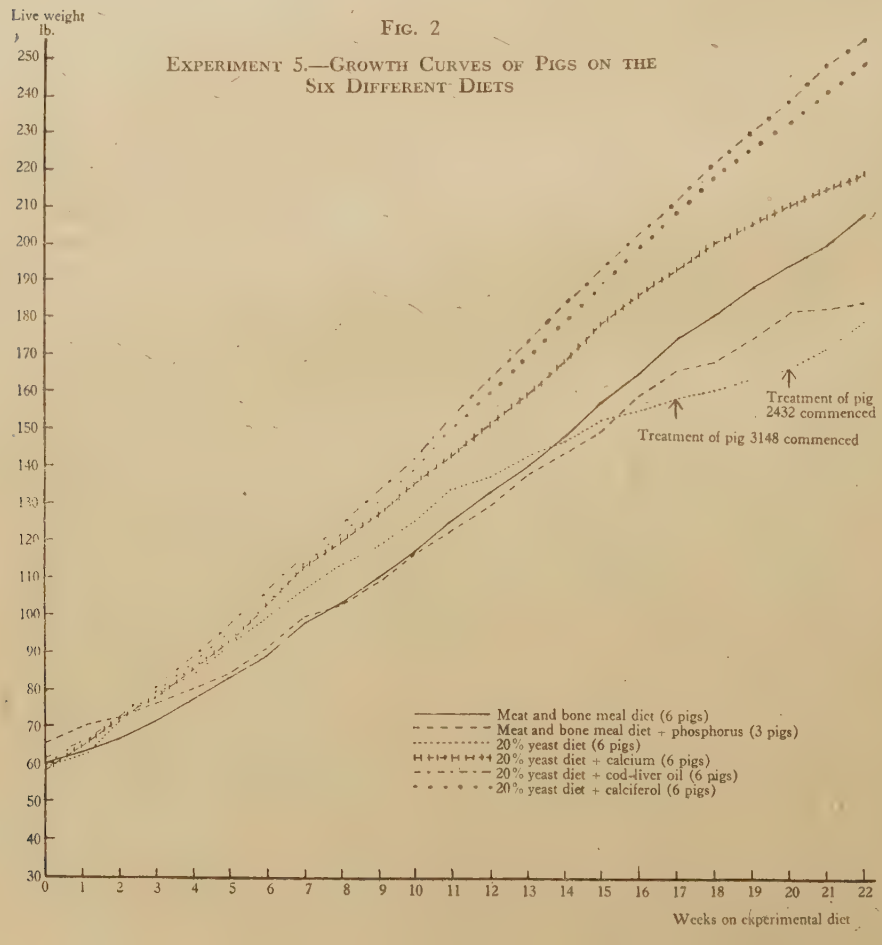
TABLE IV

EXPERIMENT 5.—GROWTH OF PIGS AND FOOD CONSUMPTION

	Diet.					
	1	2	3	4	5	6
Live Weight Gain (*lb.*)						
Total	889	362	724	970	1170	1138
Average per pig ...	131·5	120·7	120·7	161·7	195·0	189·7
Average daily gain per pig	0·96	0·78	0·78	1·05	1·27	1·23
Food Consumption (*lb.*)						
Total	4,389	2,168	4,372	4,741	4,890	4,857
Average per pig ...	731·5	723·6	728·7	790·2	815·0	809·5
Food Refusals (*lb.*)						
Number of refusals—						
Possible	924	462	868	924	924	924
Observed	91	54	371	126	46	34
Approximate amount of food refused—						
lb.	71	53	462	182	22	19
Per cent. of the total given	1·6	2·4	10·6	3·9	0·4	0·4
lb. of meal per lb. live weight gain, corrected for refusals...	4·8	5·8	5·4	4·7	4·2	4·3

FIG. 2

EXPERIMENT 5.—GROWTH CURVES OF PIGS ON THE SIX DIFFERENT DIETS



torily and better than animals in other groups. Their food refusals were negligible. All six pigs receiving the unsupplemented yeast diet (diet 3) developed symptoms of rickets, which in four of the animals were very severe. Refusal of food was very marked, even in the two animals which showed only mild rachitic symptoms. Two of the severely affected pigs were given vitamin D before the end of the experiment (see later).

Much better growth was obtained with the yeast diet to which calcium carbonate was added than on the yeast diet without it, but growth was not so good as on the yeast diet to which vitamin D was added. One of the pigs on the $CaCO_3$ diet showed slight rachitic symptoms. There were considerable food refusals but not so many as on the yeast diet without added calcium. This suggests that adding large amounts of calcium gave distinct but incomplete protection from the yeast effect.

Pigs receiving the meat and bone meal diet (diet 1) did not grow as well as might have been expected, the diet being rather unsatisfactory, though it was the best that could be provided with the foodstuffs available. None of the pigs showed rachitic symptoms.

Two of the six pigs on diet 2, *i.e.*, meat and bone meal diet with 6 per cent. sodium phosphate, died after 16 and 20 days, respectively. *Post-mortem* showed acute enteritis. A third pig scoured badly and had to be removed from the experiment after 39 days suffering from prolapse of the rectum. The remaining three pigs grew poorly and food refusals were considerable but the animals did not scour and showed no symptoms or lesions of rickets. The rachitogenic action of the 20 per cent. yeast diets is thus probably not due merely to their high phosphorus content.

Blood Calcium. The serum Ca was estimated in all pigs at the beginning of the experiment and again after eleven weeks and at slaughter (Table V). The initial level had fallen after eleven weeks in the animals on diets 1 to 4, the fall being most marked with diet 3 (20 per cent. yeast). In contrast, the serum Ca of the pigs on diets 5 and 6 (yeast + cod-liver oil or calciferol) had risen. At the final sampling the average figures showed a further decline in pigs on diets 1 to 4, while the pigs on diets 5 and 6 maintained their original level. All the pigs on diet 3 which developed symptoms of rickets showed a very low blood Ca.

Vitamin A Content of Livers (see also Table V). The fairly large variations between pigs on the same diet reflect individual and litter differences. The values are in general lower than in previous experiments. This is probably connected with the lower carotene content of the dried grass used. The vitamin A reserves of the animals receiving the 20 per cent. yeast diet were lowered, as previously, and even less vitamin A was found in the livers of pigs which received this diet with a supplement of calcium carbonate. Whilst this lowering in pigs on the high yeast diet may have been due, at least in part, to the heavy food refusals, this explanation would not apply

to those which received a supplement of $CaCO_3$, since the latter showed good growth and refused little food.

Pathological Examination. (Table VI.) There was agreement between the macroscopic and histological findings and the data for

TABLE V

EXPERIMENT 5.—SERUM Ca AND VITAMIN A IN THE LIVER

Diet.	*Serum Ca (mg./100 ml.)*			*Vitamin A in Liver.*	
	Initial.	*After 11 Weeks.*	*Final.*	*i.u./g.*	*i.u. Total (hundreds).*
1	11·1 (10·2–11·6)	10·5 (7·8–11·7)	9·4 (6·2–11·6)	6·9 (1·0–11·6)	112 (15–190)
2	10·9 [3] (10·2–11·5)	9·8 [3] (7·5–11·2)	9·2 [3] (6·5–11·0)	14·0 [3] (7·2–20·5)	194 [3] (86–308)
3	10·8 (10·3–11·3)	8·9 (6·8–11·1)	7·8 [4] (6·4–8·7)	5·1 [5] (3·0–11·2)	89 [5] (47–165)
4	11·4 (10·6–12·7)	10·9 (9·3–12·0)	9·6 (7·9–11·1)	2·5 (0·5–3·8)	44 (10–71)
5	10·9 (10·4–12·3)	11·3 (10·3–12·2)	11·3 (10·6–11·9)	77·5 (45–112)	1,695 (893–2,570)
6	10·7 (9·8–11·2)	11·2 (10·6–12·2)	11·2 (10·5–11·9)	6·2 (3·6–8·6)	134 (85–185)

Note.—Figures in square brackets indicate number of animals examined where this was less than the six originally on each diet.

TABLE VI

EXPERIMENT 5.—ASSESSMENT OF X-RAYS AND MACROSCOPIC AND HISTOLOGICAL FINDINGS

Diet.	*Radiographs.*	*Macroscopic Findings.*	*Histological Findings.*	*Total Score.*	*Mean Score per Pig.**
1. Meat and bone meal...	16	12	9	37 (6)†	6·2
2. Diet 1 + 6 p.c. sod. phosphate	6	5	5	16 (3)	5·3
3. 20 p.c. yeast	22	16	18	56 (4)	14
4. Diet 3 + 4 p.c. calc. carb.	24	12	9	45 (6)	7·5
5. Diet 3 + C.L.O. ...	15	7	8	30 (6)	5
6. Diet 3 + calciferol ...	12	10	7	29 (6)	4·8

* The minimum possible for the best bone is 3, the maximum, for very severe rickets, 30.

† Figures in parenthesis indicate number of pigs in each group at slaughter.

growth, clinical symptoms and food refusals. The diets containing 20 per cent. yeast, supplemented by vitamin D either as calciferol or as cod-liver oil, produced the best bones, the assessment showing a slight improvement over the diet of meat and bone meal. Of the six pigs on a diet rich in phosphorus (diet 2) only three survived

the experiment and none of them showed rachitic lesions. Of the six pigs on the high yeast diet (diet 3) two developed very severe rickets and were treated with vitamin D; the remaining four pigs all showed evidence of rickets, the severity varying from mild rickets to lesions as advanced as any seen previously. This variation between litters was also observed in previous experiments and may be due in part to different vitamin D reserves at weaning.

Radiographs (Table VI). The assessments are in general agreement with the macroscopic and histological findings.

Treatment of Rachitic Pigs with Vitamin D. (See Table VII and Fig. 3.)

Two pigs on the 20 per cent. yeast diet which showed severe rachitic symptoms were given calciferol in the food (3780 i.u. daily, dissolved in 14 g. of arachis oil).

TABLE VII

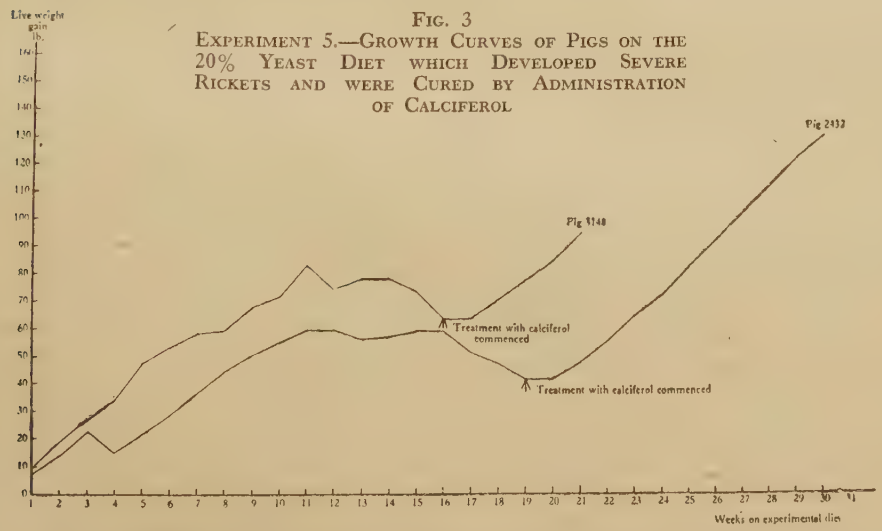
EXPERIMENT 5.—CURE OF RICKETS BY CALCIFEROL

		Number of Weeks on Diet.						
		0	11	16	19	22	25	30
Pig 2432	Serum Ca (mg. per cent.)...	10·3	6·8	—	5·7*	—	11·8	9·7
	X-ray assessment	—	—	—	10*	—	5	2
Pig 3148	Serum Ca (mg. per cent.)...	11·1	9·3	7·5*	—	11·0	—	—
	X-ray assessment	—	—	10*	—	7	—	—

* Commencement of treatment.

FIG. 3

EXPERIMENT 5.—GROWTH CURVES OF PIGS ON THE 20% YEAST DIET WHICH DEVELOPED SEVERE RICKETS AND WERE CURED BY ADMINISTRATION OF CALCIFEROL



Pig 2432 had been on the high yeast diet for 19 weeks and was showing very severe rickets. After eleven weeks' treatment it was almost normal.

Pig 3148 showed severe rickets after 16 weeks on the high yeast diet. When first given calciferol this pig had to be hand fed with a half-and-half mixture of the yeast diet and diet 1 since it refused to eat the yeast diet alone. After only eight days' treatment the animal was able to rise and feed from the trough and readily consumed the original yeast mixture. At the end of six weeks' treatment the pig was clinically normal and was slaughtered.

SUMMARY

The results are in general agreement with those of our previous work. The rachitic nature of the changes produced by feeding a 20 per cent. yeast diet has been confirmed. Rickets was prevented by addition to the yeast diet of either cod-liver oil or calciferol (1190 i.u. of vitamin D per day) and was rapidly cured by larger doses of calciferol: it was almost completely prevented by addition of 4 per cent. of calcium carbonate. When 6 per cent. sodium phosphate (Na_2HPO_4. 12 H_2O) was added to a normal diet containing meat and bone meal, rickets failed to develop, but three of six pigs died after two to three weeks with gastro-enteritis. The survivors showed no evidence of rickets and it thus appears that the rachitogenic effect of yeast is not due merely to its high content of available phosphorus. The exact mechanism of the production of rickets in pigs on a high yeast diet remains unknown.

ACKNOWLEDGMENTS

The authors are again indebted to all those who helped with the previous experiments and to whom acknowledgment was then made (Braude, Kon and White, 1943).

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Errata. Braude, Kon and White (1943), *J. comp. Path.*, **53**, 161.

p. 162. 15th line from bottom. Insert after " . . . flaked maize," " 10 parts of meat meal, . . ."
Fig. 6. For " (as Fig. 7) " read " (as Fig. 5)."
Fig. 8. For " to compare with Fig. 9," read " to compare with Fig. 7."
Table IV. Footnote. For " Table III " read " Table II."

OBSERVATIONS ON THE PATHOLOGY AND HISTOLOGY OF GRASS SICKNESS IN HORSES

By

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INTRODUCTION

Much work has been done in this Institute during the last 20 years on the histo-pathology of grass sickness in horses. No details have been published, largely because the findings have been negative. This paper records our more recent investigations and it confirms the unpublished findings of previous workers here. A general review of the disease has been given by Greig (1942).

PATHOLOGICAL AND HISTOLOGICAL FINDINGS

Histological Technique.—Tissues were fixed in 10 per cent. formol saline. For general histological examination, and possible demonstration of inclusion bodies, Anderson's haematoxylin followed by alcoholic eosin, set alight during the staining process, was used. The shorter Mann's stain was employed and for Marchi's stain the method described by Stewart (1936) was used. For other stains the unmodified routine methods were employed.

A. ALIMENTARY SYSTEM

Clinical.—A feature of the acute form of the disease is tympanites that becomes progressively more severe. Animals thus affected avoid swallowing and the accumulated saliva spasmodically drools away from the mouth. In attempting to swallow the animal may squeal, and in such cases anti-peristaltic contractions may be seen passing up the oesophagus, often followed by a trickle of ingesta from the nostrils. These symptoms have suggested a distension of the stomach, together with a continuous constriction of the pyloric sphincter. To gain information on this point, dyes mixed with melted agar were administered by stomach-pump to four horses. At autopsy the mixture was present as far as 12 feet posterior to the pyloric sphincter in one case and as far as 20 feet in the others. When carbol-fuchsin was used a piece of liver from the horse that received it gave off a red dye when immersed in formol-saline. Although autopsy findings may not be accepted as portraying the conditions that existed in the living animal, the above results do not support the assumption that the pyloric sphincter is permanently closed in this disease.

Autopsy Findings.—In horses that have died from grass sickness the main changes are found in the alimentary canal. The mucous membrane of the oesophagus may appear normal or it may show

longitudinal bands of congestion and, in some cases, ulceration. The wall is sometimes oedematous, and occasionally there is a gelatinous exudate between the oesophagus and the carotid artery and its related nerves.

In the acute form of the disease, the stomach contains an excessive quantity of liquid and is much distended with gas. The average volume of the stomach contents is about 22 litres (Stewart, Gordon and McCallum, 1940) and in some cases 30 litres has been recorded. Smith (1920) gave 18 litres as representing the capacity of the distended stomach of the horse. The contents are usually khaki-coloured and of pea-soup consistence but they may be watery and may contain fibrous material. The mucous membrane may appear normal or may show patchy congestion over its glandular portion. Sometimes, in spite of careful handling, the stomach is found to be ruptured *post mortem* and in some cases the presence of coagulated blood around the ruptured area suggests that the rupture occurred before death.

The small intestine also contains an excessive volume of fluid, commonly 30 litres, and sometimes 58 litres in amount. (Stewart *et al.*) This fluid is watery in consistence and varies in colour from yellowish-brown to greenish-brown and in very acute cases may be blood-stained. Patchy congestion of the mucous membrane of the small intestine is usually present, and there is frequently haemorrhage and oedema at the junction of the mesentery and bowel.

The large intestine usually shows impaction of varying degree and over the surface of the contents there is, invariably, a thin layer of black material in which, on microscopic examination, erythrocytes can be distinguished. In some cases drying of the contents has occurred only at the surface and the remainder of the contents appears normal, but even when the colon is not completely full and is partly distended with gas, the ingesta are found to be indented over those areas that correspond to the muscular bands of the bowel, suggesting that it has been subjected to a squeezing process. The bowel wall is dry, patchy congestion is commonly present, and the mucous membrane can be peeled off readily. The caecum shows no marked abnormality apart from patchy congestion of the membrane and the contents are of the consistence of porridge. The small colon may be empty or may contain hard, black, faecal pellets, thickly coated with mucous material.

In the chronic form of the disease, the stomach contains only one or two litres of fluid varying in consistence from that of water to that of porridge. The glandular part of the stomach may be congested. The small intestine contains up to three litres of foul-smelling, tenacious fluid of an amber or green colour. There is no congestion but the bowel has a ropelike appearance due to lack of contents accompanied by some thickening and corrugation of the mucous membrane. The large colon may be either half-filled, with material of the consistence of thick soup, or almost empty. If empty,

the organ appears abnormally small and the mucous membrane is coated with sticky mucus. The contents of the caecum are similar to those of the large colon. The small colon contains hard pellets of faeces and the mucous membrane is covered with a pasty material. Congestion of the intestinal mucous membrane is unusual in chronic cases. In affected animals destroyed and examined immediately, peristaltic waves can be observed passing over the intestinal mass. In some cases shreds of white, friable, protein-like material are passed from the rectum; in one the mass was equal in size to an orange. One piece of this material, examined histologically, showed well stained nuclei and whorls of stroma. No direct evidence is available, but it is possible that this material is exfoliated mucous membrane from the large colon.

In most cases of chronic grass sickness and in a few acute cases, the liver appears to be macroscopically normal. In the other cases it is pale and fatty or may have a mottled appearance due to areas of congestion. The pancreas and salivary glands are sometimes slightly hyperaemic, but otherwise show little abnormality.

Histological examination of the various alimentary organs adds little to the macroscopic picture. Degenerative changes are usually observed in the liver, and bacteria, agonal invaders, may be found. No infiltration of cells other than into Glisson's capsule was noted, and no inclusion bodies were observed. Except for areas of congestion or of ulceration, the mucous membrane of the alimentary tract appeared to be healthy, and even when ulceration was present, there was but little reaction in the sub-mucosa.

Detailed Histology.—(NOTE: For all tissues the numbers in brackets indicate the number of different horses from which sections were examined.)

Tongue (4), no apparent abnormality was seen. Oesophagus (5), two showed ulcerations of the mucous membrane, together with oedema of surrounding layers. Stomach (10), slight oedema in six and marked oedema in one case. Duodenum (4), two were slightly oedematous. Remainder of small intestine (6), some congestion and slight oedema in four cases, one case also showing small areas of necrosis in the mucous membrane. Caecum (4), three showed slight congestion. Colon (10), varied from normality to slight congestion and oedema; one section also showed necrosis of the epithelium and small haemorrhages.

Glands.—Parotid (5), submaxillary (5) and sublingual (5), no apparent abnormality. Liver (10), all sections were abnormal; the changes varied from cloudy swelling to marked fatty degeneration with venous congestion and cellular infiltration of Glisson's capsule. Bacilli and cocci of various sizes seen in two, believed to be agonal invaders. Pancreas (5), in all cases the secreting cells were packed with granules.

Material Extruded from Rectum.—This material shrank greatly when dehydrated in alcohol. It would stain with basic stains alone,

and these demonstrated whorls of stroma and numbers of nuclei that suggested it was an animal tissue.

B. RESPIRATORY SYSTEM

Clinical.—In affected horses respiration tends to be noisy and the respiratory rate increases as death approaches. Thus, of ten affected animals, the respiratory rate taken 24 to 12 hours before death averaged 14·2 times per minute, as compared with a rate of 14 for healthy horses in the same district. Between 12 and 3 hours before death the rate had increased to 15·9, and for five of the affected horses taken less than three hours before death the rate had increased to 32. This terminal increase in respiratory rate can be attributed to the collapse that precedes death, and suggests that respiratory upset in grass sickness is of secondary importance.

Autopsy Findings.—Lungs may present a normal appearance or vary in congestion up to a dark purple colour, in this case blood wells up when the lung is cut.

Histological examination seldom reveals any marked abnormality, although, infrequently, lung sections crumple on the microtome owing to extensive haemorrhages. Minor abnormalities may be attributed to regurgitation of food into the trachea during the last stages of the disease, to terminal anhydraemia, or to agonal invasion of the lungs by organisms, and are not pathognomonic of grass sickness.

Detailed Histology.—Naso-pharynx (5), no apparent abnormality. Trachea (5), one markedly oedematous with distortion of tissue elements. Lung (10), debris in some bronchioles in all sections examined, and slight, varying to marked, congestion was seen. Some lungs showed oedema of the alveolar walls, and in others haemorrhages, sometimes extensive, were noted.

C. UROGENITAL SYSTEM

Clinical.—In affected horses the passage of urine is infrequent, often accompanied by tenesmus, and appears to demand conscious effort. Urine passed some hours after an intravenous injection of a trypan blue solution was purple in colour, and that passed some hours after a 10 litre intravenous injection of normal saline was markedly less cloudy than previously. Passage of the catheter suggested that the vesicle sphincter was in a normal state of tonic contraction. It seems, therefore, that simple filtration from the blood is still possible and that the bladder is still under normal nervous control. It would therefore appear likely that the changes in the urogenital system are secondary in character and partly dependent on the action of the dehydrating process.

Autopsy Findings.—At autopsy the bladder is usually empty or contains a few drops of cloudy fluid ; in some cases this is due to

the passage of urine during the death agony. In one chronic case that was shot the bladder was found to be full. In the kidneys abnormality varies, ecchymosis may be present and occasionally small haemorrhages are seen, but in other cases the kidneys are pale and somewhat pulpy. The pelvis may contain a whitish, syrupy liquid, or a jelly-like material.

Histologically, the kidneys vary from a state of cloudy swelling to a marked nephrosis in a few cases. The bladder, and the uterus and ovaries in mares, appear normal. Our records do not include an autopsy on a pregnant mare.

Detailed Histology.—Kidney (10), nephrosis varying from cloudy swelling to necrosis with hyperaemia and sometimes haemorrhage. Bladder (5), ovary (5), and uterus (4), no apparent abnormality.

D. NERVOUS SYSTEM

Clinical.—Affected animals stand in a state of lethargy broken at times by periods of restless perambulations. Fine, rapid muscular twitchings, especially well seen in the region of the upper forelimb, are usually present and may, or may not, be primarily nervous in origin. Greig (1928) postulated that the majority of the clinical signs in grass sickness (*e.g.*, sweating, increased pulse rate and constipation) could be accounted for by the assumption of a state of sympatheticotonia, and for this reason vagus and sympathetic nerves and sympathetic ganglia were examined for evidence of pathological change.

Autopsy Findings.—In no case of grass sickness was any macroscopic abnormality of brain, spinal cord, peripheral nerves, or ganglia detected at *post-mortem* examination.

Histologically, no abnormality could be detected by the haematoxylin and burnt off eosin staining method. There was no abnormal grouping or clumping of nuclei that could suggest gliosis involving proliferation of astrocytes, perivascular cuffing, or other cellular inflammatory change. No inclusion bodies were observed in neurons or ependymal cells. In several cases very small haemorrhages were found in the floor of the fourth ventricle in the region of the nuclei of the dorsal motor vagus and hypoglossal nerves; but similar extravasations have been noted in the brains of horses affected with other diseases. Slight capillary hyperaemia of the cerebral cortex was noted in one case, but unaccompanied by inflammatory reaction or cell damage. This was not regarded as pathologically significant.

A small amount of lipoid material in the form of lipofuchsin is normally present in certain nerve cells, and appears in the form of yellow-orange granules when stained by scarlet red. These lipofuchsin granules were found in many cells—ganglion cells, cells of the cranial nerve nuclei, neurons of the reticular formation of the medulla and pons, but never in amounts that appeared to be abnormal considering the age of the animal from which the tissue had been

obtained. In one case compound granular corpuscles, usually in close relation to a capillary vessel, were seen in the cerebral cortex in numbers that suggested abnormality, but in no case did the amount of lipoid material suggest the presence of a lesion. Degeneration of myelin into fat droplets was not found in any case.

In a further effort to demonstrate myelin degeneration, sections of pons, medulla, vagus, splanchnic and phrenic nerves were subjected to a Marchi routine. In some cases it was noted that occasional myelin sheaths stained irregularly greyish-brown (as opposed to the jet black colour of true fat) by this method. There was, however, no fragmentation of the sheath, and, above and below the brown myelin shaded gradually into the pale brownish-yellow of " normal " myelin. These brown patches were not considered to be evidence of a pathological process and have been noted in the vagi of healthy horses destroyed by intravenous injection of chloral hydrate. Brown granules of lipofuchsin were observed in the cytoplasm of many nerve cells, including ganglion cells. No acid-fast organisms were revealed. Sections of mid-brain, pons and medulla were stained by Loyez's method, but no long standing degeneration was detected. Sections of ganglia and medulla, stained by Bielschowsky's method to demonstrate axis cylinders and neurofibrils, revealed no abnormality.

In brief, no histological abnormality specific for acute or chronic grass sickness was detected in the brain, spinal cord, sympathetic ganglia, vagus, splanchnic or phrenic nerves.

Detailed Histology.—Olfactory lobe (4), no apparent abnormality. Frontal cortex (5), Parietal cortex (5), and Hippocampal cortex (5), capillary hyperaemia in the different parts of one brain. Occipital cortex (5), capillary hyperaemia in one and a very small haemorrhage in another. Basal ganglia (5), in one case a number of small haemorrhages in the region of the substantia perforata anterior. Mid-brain (5), capillary hyperaemia in one case. Cerebellum (5), capillary hyperaemia with occasional small haemorrhages in one case. Pons (5), in three cases hyperaemia of vessels in the floor of the fourth ventricle. Medulla (10), in seven cases there was hyperaemia in the region of the ventricle floor and small haemorrhages in the region of the dorsal part of the median raphe, of the dorsal motor vagus and hypoglossal nuclei, and of the solitary tract. Cervical cord (3), Thoracic cord (5) and Lumbar cord (3), no apparent abnormality. Vagus nerve (5 ; right and left), in one case hyperaemia of the endoneurium was observed. Splanchnic nerve (3) and Phrenic nerve (1), no apparent abnormality. Thoracic ganglion (3), no apparent abnormality. Coeliac-mesenteric ganglion (5), in one case fat granules seen in a number of ganglion cells suggested some cellular breakdown ; other staining methods confirmed the apparent degeneration of isolated cells. Jugular ganglion (1) and Spinal ganglia (3 ; cervical, thoracic and lumbar), no apparent abnormality.

E. CIRCULATORY SYSTEM

Clinical.—In horses affected with acute grass sickness the pulse is fast and usually strong, but as death approaches it may become less frequent, and may lose strength until it is almost imperceptible. Average pulse rates for six horses affected with acute grass sickness were: 24 to 12 hours before death, 101; 12 to 3 hours before death, 94; less than 3 hours, 92. The average for unaffected horses in the same district was 53. In two horses in which the onset was acute but which later lapsed into the chronic form, the initial rates were 80 and 60, and these fell to 50 and 40 before death. A jugular pulse is sometimes seen in acute cases.

Autopsy Findings.—Engorgement of the spleen and anhydraemia are always present but other signs of circulatory upset appear to be dependent on a general state of toxaemia rather than to localised specific lesions. Ecchymosis may appear anywhere along the intestine or in the mesentery or along the coronary vessels of the heart. Haemorrhages, rarely extensive, may be seen in the mesentery, particularly at its junction with the small intestine, in the lungs, kidneys, adrenal glands, brain and under the endocardium; in this position they are similar to those seen in sheep dying from *Cl. welchii*, Type D intoxication (Gordon, 1934). On a few occasions there exists a thickened gelatinous patch, together with ecchymosis and haemorrhage. These patches have been noted usually at the junction between the mesentery and the small intestine but also around the oesophagus, the stomach and duodenum, between the ileum and the large colon and once surrounding one kidney.

Microscopically, no abnormality has been observed in the heart, the carotid artery or the many small vessels present in sections of other tissues.

Detailed Histology —Heart muscle (10), slight oedema in two cases, one case showed small haemorrhages throughout the muscle, another showed well-marked subendocardial haemorrhage. In all, the nuclei stained well and the muscle striations were commonly clearly visible. Carotid artery (5), no apparent abnormality. Blood clot from heart (1), considered to be an agonal clot, no apparent abnormality.

F. ENDOCRINE ORGANS

Clinical.—As the anhydraemia occurring in acute grass sickness suggested an upset in water-balance, a more extensive search was made of the endocrine glands.

Autopsy Findings.—Adrenal glands exhibited their normal yellow colour but haemorrhages, especially in the cortical zone, were often observed in the cut organ. The thyroid gland sometimes appeared to be distended with colloid; no abnormality was noted in the parathyroid and no examination of the parathyroid in a chronic case was carried out. The pituitary gland varied in colour from red-brown to blood-clot red.

Histologically, all these organs appeared to contain more than the normal amount of blood but no marked difference was noted between them and glands from horses dying from other diseases.

Detailed Histology.—Thyroid (14), in each case the follicles were distended with colloid; very few active follicles were observed in any section and the glands were considered to be in a state of stagnation. Parathyroid (6), no apparent abnormality. Adrenal (20), in ten, capillary congestion and small haemorrhages were noted in the cortex, in three the bleeding was extensive, and in one necrosis of the cortical cells was evident. Bleeding was most apparent round the fusion of the zona glomerulosa with the zona reticularis. Seven sections showed no abnormality. Pituitary (8), some degree of hyperaemia was noted in the pars anterior in all cases.

G. SEROUS MEMBRANE AND FLUIDS

Autopsy Findings.—The pleura and peritoneum, other than the omentum and mesentery—mentioned under circulatory system—showed no abnormality, with one exception in which the peritoneal covering of the diaphragm and stomach was flecked with small fibrinous strands. The peritoneal fluid may amount to 200 ml. and is usually amber colour.

Detailed Histology.—Pleura, as attached to lungs (10), and Omentum (1), no apparent abnormality. Mesentery (1), congestion of capillaries.

H. LYMPHOID AND HAEMATOGENIC SYSTEM

Autopsy Findings.—No abnormality was detected in the pharyngeal or tonsillar tissues, although in one case the secretory glands lying deep to the lingual tonsil were cystic. Lymph glands are sometimes congested, oedematous, or even haemorrhagic. The spleen is slightly enlarged. Thus the weights of spleens from 25 adult Clydesdale horses affected with grass sickness varied from 2 lb. to 14 lb., with an average of 5·4 lb., whereas the normal average weight (Sisson, 1938) is about 2·5 lb. Spleens from 18 horses dying from other diseases in the same district at the same time of year ranged from 2 lb. to 19 lb., with an average of 4·3 lb.

Histologically, the lingual, pharyngeal and other lymphoid tissues appeared more or less normal; in the spleen a carpet of erythrocytes was all that could be seen with the exception of a few cases in which the normal histological features were faintly visible. The bone marrow was not examined but the circulating blood investigated by one of us (Holman, 1940) appeared to reflect an active reaction of the myeloid tissue.

Detailed Histology.—Tonsil (4), no apparent abnormality. Bronchial lymphatic glands (5), congestion of entire gland in one case. Mesenteric lymphatic glands (10), two showed oedematous changes,

the nuclei staining imperfectly and abnormally large numbers of neutrophil leucocytes present. Two other cases showed congestion. Spleen (10), in every case the normal histological structure was obscured by a mass of erythrocytes.

I. MUSCLE

Clinical.—Localised muscular twitching is seen in acute cases; in chronic cases the voluntary muscle appears hard and dry, possibly owing to the loss of water and fat from the skin and connective tissue.

Detailed Histology.—Voluntary muscle (6), the nuclei were well stained, the muscular striations were clearly visible and there was no infiltration of cells in the connective tissue.

INTRAVITAL STAINING

Intravital staining with trypan blue has been recommended for picking out damaged tissues, particularly damaged blood vessels (Cappell, 1929) and it has been used to demonstrate virus lesions on the chorio-allantoic membrane (Cooke and Blattner, 1942). It seemed possible, therefore, that intravenous injection of this dye into affected horses might reveal areas of increased capillary permeability or the effects of a virus. In a similar manner, Higgin's ink (containing fine carbon particles) has been used to demonstrate either anachoresis or capillary permeability. Both these dyes were used for intravenous injection into the jugular veins of horses affected with grass sickness.

Trypan blue, in watery solution, was given to affected horses intravenously in doses up to 30 g. Other horses received an intravenous injection of Higgin's ink up to a maximum dose of 500 ml. Trypan blue in small doses could not be identified with certainty in the tissues after death, but at autopsy, following a 15 g. dose, the liver was slatey-green, and khaki coloured on section, the heart valves, venae cavae, aorta and pulmonary vessels were blue and the buffy coat of the heart clot was bright green. After a 25 g. dose of trypan blue the right lobe and diaphragmmatic border of the liver appeared blue, the glandular area of the stomach had a greenish tinge, the heart was bluish throughout with the auricles, great vessels and endocardium of the left ventricle more deeply stained. On section the middle coat of the oesophagus was found to be blue and the pleura surrounding the oesophagus in the thorax was streaked with blue. Connective tissue covering the carotid artery and the large nerve trunks was also tinged with blue, and the articular surfaces of the femur and other long bones were of a grey-blue colour. The urine was stained a deep purple 13 hours before death and at the time of death.

A 30 g. dose of trypan blue was injected into a horse affected with chronic grass sickness. At ***post mortem*** in this case, fascia, the

articular surfaces of the long bones, the lining endothelium of the auricles of the heart, and the connective tissue covering of the coronary vessels were stained blue. The liver was bluish-green and on section a darker green. The glandular part of the stomach had a light, leaf-green hue, while the cardiac stomach, mucous membrane of the small intestine and large and small colons had a jade green tint. On section, the renal cortex was greenish-blue and the renal medulla red. The urine was of a dark purple colour.

Histological examination of all the stained tissues as detailed above, with the exception of the articular surfaces of the long bones, was carried out but failed to reveal the dye.

Higgin's ink was not observed macroscopically after death in the organs of horses to which it had been administered, although in two cases out of three the liver, on section, had a greenish tinge. Histologically, however, dark-green granules lying in the sinusoids of the liver and within the cytoplasm of Kupffer cells were found in every case. In one case pigment was also found in the dust-cells of the lung and also lying, apparently free, in the inter-alveolar tissue. In no cases was there localised seepage of dye indicative of the presence of leukotaxine.

SUMMARY

This paper presents the autopsy picture of grass sickness in horses as recorded in examinations made over a number of years. Included in the description are details of histological examinations made on the following sets of tissues: (*a*) alimentary tract and its digestive glands; (*b*) respiratory tract; (*c*) urogenital system; (*d*) nervous system; (*e*) circulatory system; (*f*) endocrine glands; (*g*) serous membranes; (*h*) lymphoid and haematogenous system; (*i*) muscular system. No lesions, foci of bacteria or cells showing inclusion bodies were detected in any of these tissues.

Little fresh positive information has been added and the outstanding features at autopsy remain as follows. In acute types of the disease the stomach is distended by the presence of gas and fluid, an excessive amount of fluid is also present in the small intestine and, with few exceptions, the colon is impacted and its contents are covered with a black coating that contains blood. Patchy congestion of varying degree is commonly seen along the length of the alimentary tract, together with ecchymoses in parts of the mesentery; toxic changes are usually present in the liver, kidney and lungs; sub-endocardial haemorrhages in the heart are frequently observed in horses left to die. The general picture in chronic cases is one of inanition and little congestion is evident. The alimentary tract is nearly empty and the faeces within the rectum consist of hard pellets, coated with mucus.

In both acute and chronic cases the adrenal gland often shows small haemorrhages—as in other toxic or infectious conditions—and the spleen is packed with red cells—as in fatal cases of colic.

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ACUTE AND SUBACUTE TRYPANOSOMIASIS IN CATTLE CAUSED BY *T. VIVAX*

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INTRODUCTION

Although the acute form of trypanosomiasis caused by *T. simiae* in pigs has now been observed in the majority of tropical African territories, it would appear that no cases of similarly acute trypanosomiasis in cattle have been recorded. Bevan (1928) referred to what he called acute cases which were encountered on some occasions "especially in the rainy season when cattle die suddenly although perhaps in good condition." Possibly Bevan's cases may have been sudden fatal relapses in animals that had been infected for some time. We have recently received from the field reports of a rapidly fatal, fulminating type of infection in cattle with *T. congolense* or *T. vivax*, swarming in the blood, which under the circumstances may have been true acute cases, running a course comparable to that seen in *T. simiae* infection of pigs.

In the course of experiments with various chemotherapeutic agents we have seen a number of cases of infection in cattle, either with pure *T. vivax* or with a mixture of *T. vivax* and *T. congolense*, in which the course of the disease has been exactly similar to that of *T. simiae* infection in pigs. It is possible that the unusually acute character of the disease is to be ascribed in all cases to the presence of *T. vivax* since no such cases have occurred in the laboratory with pure infections of *T. congolense*. That the rapidly fatal termination is solely attributable to the trypanosome seems evident, since cases have occurred with three different strains of *T. vivax*, and since the pathology of the disease is so similar to that of *T. simiae* infection in pigs.

OCCURRENCE

All the cases have occurred in cattle infected by the subcutaneous injection of blood. The three strains of *T. vivax* were obtained from natural cases in cattle at Emali, Solai and Machakos.

With the Emali strain of *T. vivax* the first passage animal died from normal trypanosomiasis on the 28th day. Of two cattle subinoculated from this animal and left untreated, one died of acute trypanosomiasis on the 19th day and the other of subacute trypanosomiasis on the 26th day.

With the Solai strain the first passage animal died with lesions of the chronic type in 103 days. Three animals were inoculated from this calf and left untreated. Of these, one died of acute trypanosomiasis on the 14th day, another of subacute infection on the 31st day and the third appeared to make a natural recovery. In

the third passage one animal was left untreated and died of the usual chronic form of the disease on the 83rd day. In the fourth passage the one untreated animal died after 30 days with lesions of the subacute form.

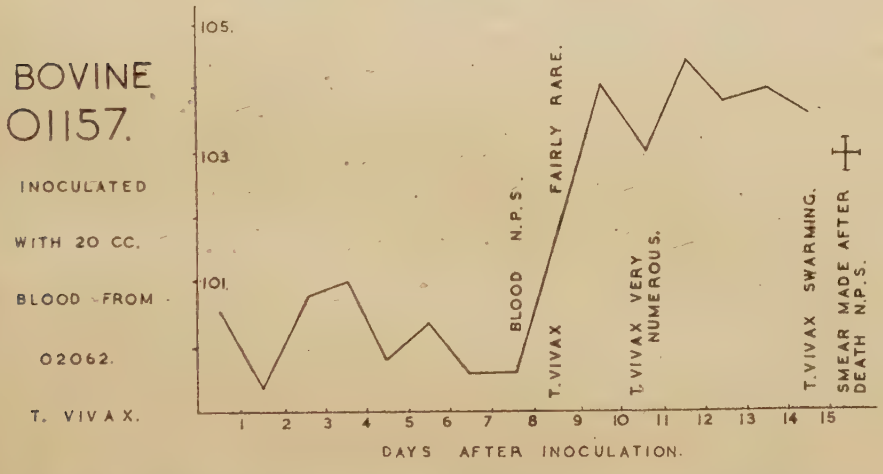
With the third strain, of three animals in the second passage which were not treated, one developed the acute form, dying in 29 days, one the subacute form dying in 34 days, and one the chronic form dying in 70 days.

In addition to the above pure *T. vivax* cases, three acute cases occurred in animals inoculated simultaneously with the second passage Emali strain and a strain of *T. congolense*. These three animals died in 15, 19 and 20 days, respectively.

SYMPTOMATOLOGY

The symptoms and pathological lesions of these acute cases are those of a septicaemia. The incubation period may vary from 4 to 20 days but is usually under ten. The onset of the disease is sudden (see Chart I), the temperature shooting up to 104° F. or higher. There is high fever for a period, possibly a week, and death may occur

CHART I.



during the period of pyrexia or the temperature may fall suddenly to several degrees below normal. The animal goes down, is unable to rise, and death is preceded by coma. Apart from those associated with the pyrexia, there are no obvious symptoms.

PARASITES

Whereas in chronic bovine trypanosomiasis it is unusual to find trypanosomes in great concentration in the blood, in these acute cases the term "swarming" aptly describes the numbers usually present towards the end of the temperature reaction. Smears taken

shortly before or after death may show parasites in numbers; but are frequently negative. Large numbers of parasites are, of course, often seen in smears from laboratory rodents infected experimentally with, for example, *T. brucei*; but among domesticated mammals the swarming of *T. simiae* has always been considered somewhat unique.

PATHOLOGY

An animal in good condition at the time of infection will usually retain its condition up to death, for death occurs before the depletion of bodily reserves can proceed very far. On the other hand, an animal in poor condition contracted the acute form and died in 14 days, hence good condition is not a prerequisite for the development of this form.

The parietal peritoneum and the serous surfaces of the stomach, intestines and other abdominal organs are usually covered with haemorrhages, up to 4 mm. in diameter. There is no excess of fluid in the cavity.

Small submucous haemorrhages are frequently present along the sides of the tongue and on the underside of the free portion. Similar haemorrhages are constantly present in the larynx, and a few may be found in the pharynx. The folds of the abomasum are usually peppered with pin-head-sized haemorrhages, and larger ones, up to 8 mm. in their larger diameter, are present on the pyloric portion. Some of the larger haemorrhages, on occasion, show a tendency to ulcerate. The duodenal mucosa is peppered with small discrete haemorrhages, which may extend to the anus, although a number of cases show no lesions in the colon and rectum. In a few animals free haemorrhage had occurred into the lumen of the caecum, the contents of which contained fresh and changed blood.

The liver is always enlarged, sometimes markedly. The surface is mottled and multiple fine haemorrhages may be visible. In some cases there is generalised jaundice, when, of course, the liver has a definitely orange tinge. The gall-bladder may show subserous or submucous haemorrhages or both, and the bile is often yellowish-brown and grumous.

Discrete haemorrhages are usually found in the perirenal fat. The kidneys are generally pale, with fine haemorrhages on the surface of the cortex, and on section *similar* haemorrhages will be found throughout the organ. The mucosal surface of the bladder is frequently peppered with haemorrhages, some of which may be as much as 8 mm. across. The urine is clear and varies in colour within normal limits, but may be dark in jaundiced cases.

The parietal pleura is usually covered with fine subserous haemorrhages and similar haemorrhages cover the surface of the lungs. A few submucous haemorrhages are usually present in the trachea and discrete round haemorrhages are frequently scattered throughout the lungs. Oedema, if present, is never marked.

Very extensive extravasations and petechiae are constantly present beneath the epicardium and endocardium covering the ventricles. A few smaller petechiae may also be found on the auricles. The myocardium appears degenerated, and sometimes contains obvious diffuse haemorrhages. The spleen varies in size up to twice the normal. The capsule usually shows haemorrhages and the Malpighian corpuscles are often considerably swollen. The lymph glands are normal in size or only slightly swollen; but when cut they are usually congested and show numerous haemorrhages throughout the section.

No marked lesions are visible in the brain. The cerebrospinal fluid appears increased in quantity; but is normal in appearance.

Remarkable lesions are frequently seen in the abdominal muscles and in the muscles behind the shoulder. These consist of black areas resembling an anaerobic lesion but free from gas. Pinhead haemorrhages have been seen in the fascia overlying the ribs. There is no general oedema; but jaundice may be present in animals which have *no* concomitant infection with *Babesia* or *Anaplasma*.

PATHOLOGICAL HISTOLOGY

A complete histological study has not been attempted; but lesions have been found in the liver, adrenals and anterior pituitary. The sinusoids of the liver are dilated, and both sinusoids and central veins show an excess of white-cell nuclei, sometimes polymorph and sometimes of small-round-cell type. The Kupfer nuclei are slightly swollen but not active. The cells of the parenchyma may be packed with yellow pigment, and there may be areas of cells with condensed cytoplasm and shrunken, deeply stained nuclei adjacent to, but not necessarily surrounding, the central veins. One liver showed irregular small areas in which the cytoplasm was vacuolated and partly necrosed, and in which the hepatic-cell nuclei were undergoing karyorrhexis.

The adrenals of two animals have been sectioned and in both there is marked disorganisation of the zona glomerulata of the cortex. The cell groups appear separated and some cells are even free. Presumably this change is due to oedema. The zona fasciculata is more normal; but in neither animal are oxyphile granules present. One adrenal shows small foci of what appear to be young organ cells and small round cells. The anterior pituitary is abnormal in that the cytoplasm of the oxyphile cells is packed with granular, weakly eosinophilic material, the usual bright discrete granules being absent.

BLOOD CHEMISTRY

The original observation of Yorke, Adams and Murgatroyd (1929) that trypanosomes use up really large quantities of glucose *in vitro* has been confirmed by a number of investigators. In general, pathogenic trypanosomes consume more glucose than non-pathogenic

forms, and the amount of glucose metabolised is out of all proportion to the size of these parasites when compared with other species. Much work has also been done on the possible effect of the nutrition of the parasites on the sugar metabolism of the host, but the results have been rather contradictory. Some authors have reported no change in the blood sugar level, others have found a hyperglycaemia during part of the course of the infection; but the majority of workers have demonstrated a hypoglycaemia, at least during the last few days or hours of a fatal infection. Schern (1925, 1927) attempted to explain the pathogenic action of trypanosomes by their effect on carbohydrate metabolism.

TABLE I

BLOOD ANALYSIS OF BOVINE 1157

Inoculated 20.1.42 *with Blood of Bovine* 02062 (*pure T. vivax*)

Days After Inoculation	0	7	8	9	10	11	12	13	14	15
Temperature, °F. a.m. ...	100·4	100·6	101·6	104·0	103·0	104·4	103·8	104·0	103·6	Died
R.B.C. in millions/mm.^{3} ...	3·94	4·00				3·30		2·36		
W.B.C. in thousand/mm.^{3} ...	6·55	7·3				5·60		3·15		
Haemoglobin in g./100 c.c. ...	6·3	5·6				5·5		4·8		
Inorganic P mg./100 c.c. ...	7·12	5·14				—		3·34		
Blood glucose, mg./100 c.c. ...	41	36				36		<20		
Blood urea, mg./100 c.c. ...	13·8	—				24·6		36		
Serum N.P.N., mg./100 c.c. ...	20·6	29·6				—		37		
Blood chloride, mg. Cl/100 c.c.	326·2	305·8				317·5		310·6		
van den Bergh ...	—							++		
Parasites ...	0	0	+		+++				Swarming	After death 0

Haemoglobin was determined with a colorimeter using a Newcomer glass standard disc.
Blood glucose was determined by the Shaffer Hartmann method on a Somogyi filtrate.
Blood urea was determined by Scotts' method (*Brit. J. exp. Path.*, **21**, 93).
Serum N.P.N. was determined by the method of Folin and Wu.

The effect of *T. congolense* and *T. brucei* on carbohydrate metabolism in the domesticated herbivora has been discussed recently by French (1938b). He points out that the number of parasites found in the blood, even at death, is small, and that swarming, as observed in small experimental animals, does not occur. Hornby (1931) compared the effect of *T. congolense* on cattle with the effect of repeated bleedings. Certainly in its usual chronic form, *T. congolense* infection of cattle is essentially an anaemia. The experiments suggest, moreover, that the anaemia is caused by the elaboration

by the parasites of a haematopoietic inhibitor. In French's own investigations a sharp fall in blood sugar occurred shortly before death or crisis ; but the data suggest that this fall is not directly related to the consumption of glucose by the parasites.

A study of the blood sugar in acute cases of *T. simiae* infection in pigs and the acute cases of trypanosomiasis observed in cattle, on the other hand, certainly suggests that there is a connection between " swarming " of the parasites when this phenomenon is seen and the remarkably low values for blood glucose. Death may occur before the development of any marked degree of anaemia, and whilst no claim is made that death is due to glucose starvation, these acute cases are characterised biochemically as those cases in which there is swarming of the parasites followed by a severe drop in blood sugar values.

A complete study of the blood changes in these acute cases in cattle is rendered difficult by the fact that in any series of transmissions they occur almost fortuitously. Actually, we have a complete record (Table I) of only one animal, and the results are unfortunately complicated by the fact that this animal had recently recovered from attacks of redwater and anaplasmosis. Figures are, however, available showing the results of two or more blood examinations of two other animals during the last few days of the acute disease, of three animals which were treated and of one animal which died on the 26th day, and in which lesions of the acute form were absent. (Table II.)

The tables show that animals 1157, 0675 and 0679 developed a severe hypoglycaemia shortly before death, which in each case occurred during the temperature reaction. Cattle Nos. 0674 and 0682 also show a very marked fall in blood sugar, the former during the reaction and the latter during the period immediately after the temperature had returned to normal. In the first three animals the hypoglycaemia was associated with the presence of large numbers of trypanosomes in the blood and in 0674 parasites were very numerous at the time when the lowest blood glucose value was obtained. In 0682 parasites were never so numerous and the blood glucose never fell too low to be determined by the method used.

Bovine 1070 was exceptional in that in spite of the fact that *T. vivax* were numerous on the 16th day after infection, the blood glucose value never fell to a truly low figure. In bovine 0673 a terminal hyperglycaemia developed shortly before death ; but this animal did not show the typical lesions of acute trypanosomiasis.

A fall in the red cell count was observed in all the cattle. Owing to the protozoal infections prior to inoculation, 1157 was anaemic from the start, and of the other typical, fatal cases 0675 had a dangerously low figure at the time of death. It seems certain, however, that the death of 0679 cannot be attributed to anaemia.

Evidence of a negative kind is provided by 0673. In this animal the lowest blood sugar value found was 31 mg. ; but the red cell

TABLE

BLOOD ANALYSES

Days after inoculation	11	12	13	14	16	19	20
0675 *Mixed T. vivax—T. congolense*							
Temperature, °F. a.m.	102·0	103·2	104·8	103·2	105·2	104·0	—
R.B.C. in millions/mm.^{3}					5·56	2·56	
Blood glucose mg./100 c.c.					41	<20	
Parasites	0			0	+++ (mixed)	After death +++	
0679 *Mixed T. vivax—T. congolense*							
Temperature, °F. a.m.	101·8	104·2	105·4	104·0	104·8	103·0	102·0
R.B.C. in millions/mm.^{3}					7·26	5·1	
Blood glucose mg./100 c.c.					36	<20	
Parasites	0			+	+++ (*T. vivax*)	++++	After death, blood 0
1070 *Mixed T. vivax—T. congolense*							
Temperature, °F. a.m.	104·0	104·8	106·6	104·4	104·4	105·0	101·8
R.B.C. in millions/mm.^{3}					4·90	3·44	3·6
Blood glucose mg./100 c.c.					65	46	46
Parasites	+				+++ (*T. vivax*)	Treated with Phenanthridinium compound	2 *T. congolense* seen

Days after inoculation	7	8	9	11	13	14	15
0674 *Mixed T. vivax—T. congolense*							
Treated 22nd day	100	100·6	100·6	103·0	100·4	104·2	102·8
Temperature, °F. a.m.							
R.B.C. in millions/mm.^{3}							
Blood glucose mg./100 c.c.							
Parasites				+			
0682 *Mixed T. vivax—T. congolense*							
Treated 22nd day	101·4	104·0	105·0	105·6	106·2	106·2	103·6
Temperature, °F. a.m.							
R.B.C. in millions/mm.^{3}							
Blood glucose mg./100 c.c.							
Parasites				+			

Days after inoculation	5	6	7	11	16	17/18	19
0673 *Pure T. vivax*							
Temperature, °F. a.m.	101·6	105·2	105·2	105·0	104·0	102·0	101·2
R.B.C. in millions/mm.^{3}							
Blood glucose mg./100 c.c.							
Parasites				+			

* On day of death this animal had 29·4 mg. urea/100 c.c. blood and 7·45 mg. inorganic and looking very sick).

II
OF OTHER CATTLE

							Remarks
							Died 19th day, 12.45 p.m. Acute case.
							Died 20th day. Acute case.
							Died 35th day—redwater.

16	17	19	20	21	22	23	*Remarks*
105·0 7·62 <20	105·0	105·2 5·56 36	105·0 5·12 46	103·0 5·06 36	101·0	100·4 4·52 46	Died 33rd day of redwater.
+++		++			Treated		No trypanosomes in smears.
102·6 8·88 26	100·8	101·8 6·24 21	104·0 5·8 31	104·0 4·65 51	103·4	101·2 4·32 41	Died 63rd day of tympanites.
+ (deformed)		+ (*T. vivax* only)			Treated		Trypanosomes did not return.

20	21	22	23	24	25	26*	*Remarks*
103·0 4·54 46	102·0	102·0	102·0 3·28 46	101·4	99·4 1·42 31	96·2 1·56 84	Died 26th day. Lesions *not* those of acute trypanosomiasis.
++					0		No parasites in blood or gland after death.

P. On the day previous to death N.P.N. was 53·25 mg./100 c.c. blood (animal down

count was below two millions on the day prior to and the day of death. On *post-mortem* examination there were none of the haemorrhagic lesions of acute trypanosomiasis ; in fact, from the lesions found, the animal might quite easily have been a chronic case.

In view of the limited number of observations it is not proposed to discuss at length the changes in the other blood constituents. The figures available suggest that the inorganic phosphorus figure is reduced but rises sharply in the agonal stage. The blood urea and serum NPN figures are, as one would expect in a febrile disease, increased. The blood chloride figures shows little significant change. (French, 1938a.)

COMPARISON WITH *T. simiae*

The acute form of bovine trypanosomiasis resembles *T. simiae* infection in pigs in its clinical course, pathology and in the blood picture at the time of death. We have not, however, seen hyperacute cases such as have occurred with strains of *T. simiae* passaged at Kabete and in which the interval between inoculation and death has been three to four days. In such cases death often occurs 30 to 50 hours after the first rise in temperature, trypanosomes are present in great numbers in the blood and it appears as if the course of the disease is too short even for the development of good haemorrhagic lesions.

In acute experimental cases of *T. simiae* infection the period between inoculation and death is usually between 5 and 12 days. The incubation period is short, lasting about two to three days ; there is then a sudden rise in temperature to 104° F. or more. On the succeeding days the temperature remains high and death occurs either before or shortly after the temperature falls. By contrast, in *T. congolense* infection in pigs there is an irregular and less severe temperature reaction, similar to, but more marked than, that usually seen in chronic *T. congolense* infection in cattle.

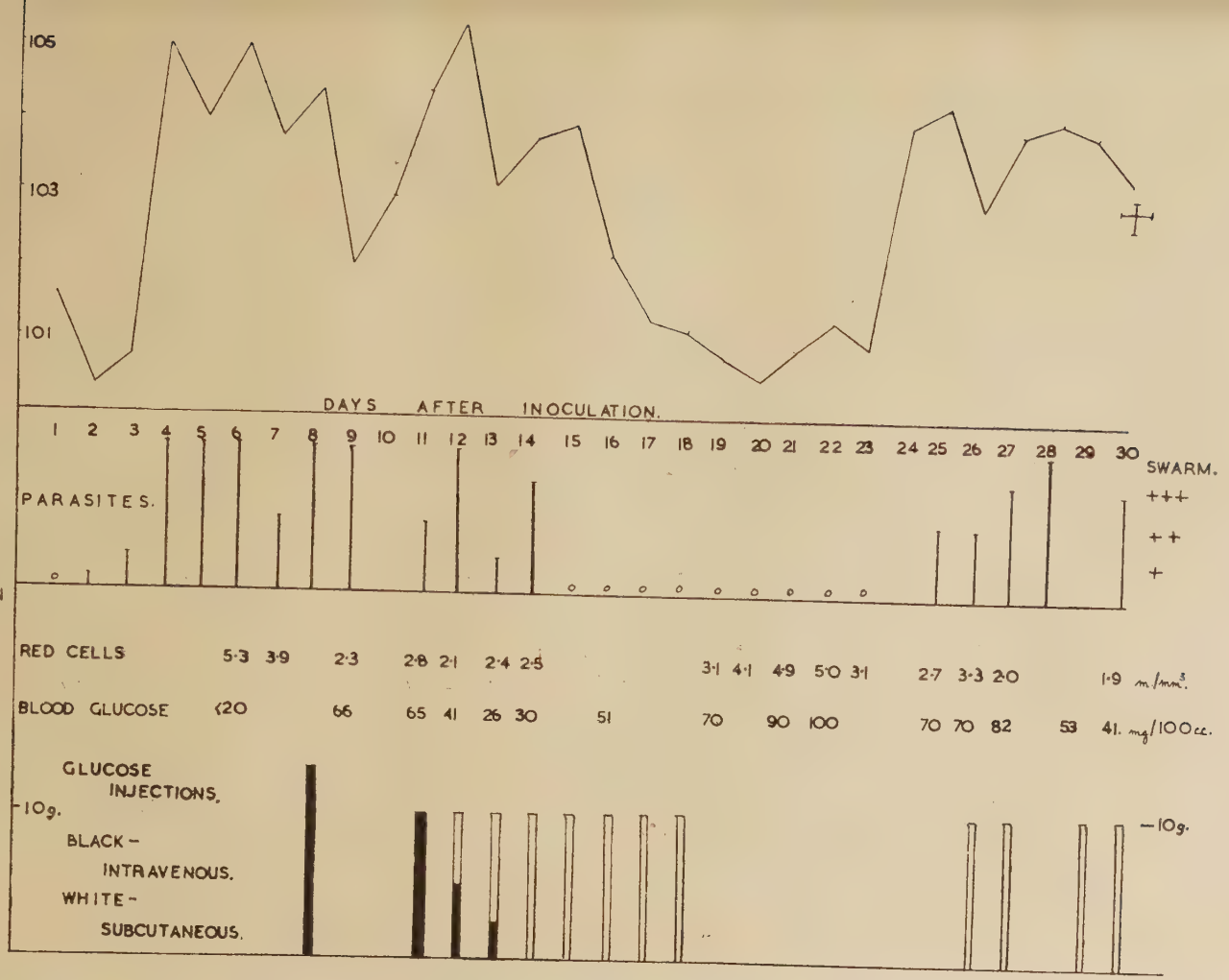
During the greater part of the temperature reaction, *T. simiae* is found in large numbers in the blood ; but it may disappear shortly before death. Death is often preceded by a short period of coma and after death the lesions found closely parallel those which have been described above in the acute cases of *T. vivax* in cattle. The carcase shows no evidence of cachexia or oedema, and haemorrhages are present on the peritoneum, in the fat depots, on the epicardium and endocardium and at other sites.

Chemical changes in the blood are also similar, in that the fall in glucose value about the time of death is very marked. The red cell count also falls, but the drop is not always marked and it is unusual for the carcase to appear anaemic to the eye.

We attempted to prolong the life of an infected pig by the administration of glucose, and we believe we were successful. The chart of this animal (Chart II) is reproduced, since, taken in conjunction with the findings in respect of glucose and red blood counts in

PIG
2162

INOCULATED
WITH
T SIMIAE
MAKUYU STRAIN
25/4/40.



acute and chronic cases of trypanosomiasis, it lends strong support to the idea that acute deaths may be attributable to glucose deprivation. Whereas no untreated pigs inoculated with this strain survived for a fortnight, it will be seen from the chart that this pig lived for 30 days from the day of inoculation. At death the lesions present were those of chronic trypanosomiasis—marked anaemia, enlarged spleen and hydropericardium. There were no haemorrhages such as characterise acute *T. simiae* cases. The chart suggests that the inoculation of glucose helped the pig to survive the first cycle of trypanosome multiplication and that when it succumbed during a later cycle the course was similar to that of the ordinary chronic disease.

We also attempted to prolong life in two cases of acute *T. vivax* infection in cattle; but possibly because treatment was started too late (until an animal refuses to stand up it is difficult to decide whether it is likely to die of the acute form), possibly because our dose of 50 g. glucose intravenously was too small, these attempts were not successful. Two animals whose blood glucose was below 20 mg./100 c.c. at 9 a.m. were given this dose and re-bled three-quarters of an hour later. The glucose level of one was then found to be 51 mg. and this animal died at 12.45. The response in the other animal was better for the sugar level was 103 mg. after the ¾-hour interval. At 3.45 in the afternoon this animal was bled again, when the glucose value was found to be 31 mg. and a further dose of glucose was administered. At 10.30 p.m. this animal looked brighter than it had done all day; but it was found dead in the morning. It is hoped that an opportunity of repeating this experiment will occur, when treatment will be attempted by giving all but the first glucose inoculation subcutaneously, so allowing for slow absorption and reducing the risk of glucose being lost through the blood level temporarily rising above the renal threshold.

SUBACUTE TRYPANOSOMIASIS

Owing to the hyperacute or acute course of the disease it has been usual to regard *T. simiae* infection of pigs as a unique form of trypanosomiasis; but the record of acute cases of *T. vivax* infection removes its claim. It has been shown that in symptoms, pathology and biochemistry there is a close similarity between the acute cases in the two infections. We have not had deaths in as short a period as three or four days following inoculation with *T. vivax*, and we consider that such cases in pigs justify description as hyperacute.

The term chronic trypanosomiasis is obviously applicable to the typical form in which animals often live for several weeks or even months after infection. The temperature reaction is not usually marked but periods of fever occur from time to time. On *post-mortem* examination cachexia and oedema are the prominent lesions.

Intermediate between the acute and chronic forms we have seen several cases similar to bovine 0673 (Table II) which have shown

a marked temperature reaction but in which death has not occurred until several days after the temperature has fallen and in which on *post-mortem* examination haemorrhagic lesions have not been prominent. These cases may be termed subacute. In animals infected with *T. vivax* alone death has occurred at periods varying from 26 to 34 days after inoculation and the evidence suggests that death is attributable, in the main, to anaemia.

After death the lesions found are rather variable. In some cases haemorrhages are present but they are by no means as numerous or widespread as in the acute cases. In various animals they have been found beneath the epicardium and endocardium, beneath the capsule of the liver and spleen and rarely in the kidney. The spleen may be slightly enlarged or normal in size and on section the cut surface is pale.

The blood is always pale and some oedema of the lungs and subcutis is usually present. The lymph glands are somewhat swollen and oedematous and show congested or haemorrhagic areas. The haemolymph glands are often prominent.

Lesions in the alimentary tract are usually confined to oedema of the serous coat of the abomasum and faint black reticular markings in the caecum. As in acute cases, jaundice may be present.

SUMMARY

1. Cases of acute and subacute trypanosomiasis of African cattle caused by *T. vivax* are described.

2. Acute cases are characterised by the extensive haemorrhagic lesions found *post-mortem* and by a marked fall in blood glucose shortly before death.

3. Subacute cases may be described briefly as those in which the course is rapid but in which death is associated with severe anaemia rather than with a low blood sugar value.

4. The cases described affect our appreciation of the unique status of *T. simiae* infection in pigs since it is shown that analogous cases occur in cattle infected with *T. vivax*.

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STUDIES ON THE SURVIVAL OF JOHNE'S BACILLI

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INTRODUCTION

Johne's disease of cattle is believed to be spread mainly by the contamination of water, foodstuffs and pasture land with infected faeces. Johne's bacilli probably survive for long periods outside the animal body and this adds to the difficulty of the control of infection. This longevity of the bacillus is assumed because of the general characteristics of the organism and its similarity with the tubercle bacillus which is known to survive for long periods.

Maddock (1933, 1934, 1936) showed that some virulent tubercle bacilli of the bovine type survived six months' exposure in soil, soil and dung mixtures, and in dung. On growing grass, similar bacteria remained alive and were virulent for upwards of 63 days. There were, as expected, some variations in different years and seasons. Schalk (1928) worked with tubercle bacilli of the avian type and found that these remained alive in a contaminated barnyard for at least five months. Buried carcases of tuberculous poultry remained infective for from three to 12 months. Whilst these studies are useful and instructive there are several reasons why they should not be accepted as being necessarily applicable to Johne's bacilli nor should they form any basis for measures taken to control Johne's disease. Johne's disease and tuberculosis differ in many respects and the techniques for the recovery of the respective bacteria are not entirely comparable. In well-established cases of Johne's disease the faeces are constantly and often heavily infected with the causal organism. In tuberculosis of poultry this may also obtain but in bovine tuberculosis the excretion of living tubercle bacilli in the faeces is variable and in many cases is probably from swallowed infected sputum; Williams and Hoy (1928) recovered tubercle bacilli only intermittently from the dung of naturally infected cows. Tubercle bacilli may be recovered from infected material by culture and by the use of experimental animals; Johne's bacilli can only be isolated by cultural methods. The question of the viability of Johne's bacilli is therefore a specific one and ought to be treated as such. The problem has been studied by Vishnevskĭĭ *et al.* (1940) who used one strain of Johne's bacillus which they mixed with bovine faeces and soil and recovered it after eleven months. They were not able to isolate it seven days after it had been added to urine. The strain used was clearly one which had been trained to grow on media which did not contain extracts of other acid fast bacilli and their results may therefore not be applicable to freshly isolated ones.

The available data on the problem are scanty and it is clearly important that some knowledge should be obtained concerning the viability of Johne's bacilli especially after exposure under natural conditions. A series of experiments designed to test the survival of bacilli under various conditions were made and are described under three headings :—

(A) The survival of cultures of bacilli in different kinds of sterilised water kept in a cupboard in the laboratory.

(B) The viability of Johne's bacilli when intestinal scrapings from natural cases of disease were suspended in unsterilised river water and kept in the open air.

(C) The viability of Johne's bacilli in naturally infected faeces after exposure to ordinary weather conditions.

The technique in each series of experiments varied slightly but in general it consisted of removing samples at intervals and culturing them after treatment with antiformin. The culture medium consisted of an egg mixture with 25 per cent. liver extract and an extract of *Mycobacterium phlei*; this is similar to one used by Dunkin (1928) with no dye. After antiformin treatment the washed centrifuged deposits of the samples were inoculated on the surface of slopes of media with capillary pipettes and incubation at 37° C. was prolonged, if necessary for three to four months. Representative cultures from each sample were tested for their ability to grow on the modified Dunkin's medium and their inability to develop on egg, glycerine egg, and agar slopes. Their morphological and staining characteristics were also studied and these tests were rigidly adhered to before accepting a culture as that of Johne's bacillus. No tubercle bacilli were isolated from any of the samples of intestinal scrapings or faeces although guinea-pigs were injected with suspensions of some; this was done to prevent confusion which would occur had they been present.

The brief details of the tests and the results obtained are recorded below.

(A) SURVIVAL OF JOHNE'S BACILLI IN STERILISED WATER AT ROOM TEMPERATURE

The viability of cultures of Johne's bacilli was tested in distilled water, tap water, and pond water containing dried mud, and accordingly nine bottles were prepared as follows:—(*a*) Three with 500 c.c. of distilled water; (*b*) three with 500 c.c. of tap water; (*c*) three with 500 c.c. of pond water, each containing 25 g. of dried mud. The bottles containing the water were then sterilised by heat and rubber bungs inserted. An emulsified saline suspension of a 39 days' growth of a culture of Johne's bacilli obtained from the mesenteric gland of an infected cow was prepared and the amount calculated in milligrams of moist bacilli. The following amounts were added, respectively, to the three bottles of each series, 17 mg. 6·4 mg., and 0·525 mg. The contents were shaken and kept at room temperature. Two 10 c.c. samples were removed from each bottle at monthly intervals and after centrifugation the deposit from one sample was inoculated on to two tubes of media; the deposit from the other sample was treated with 10 c.c. of 10 per cent. antiformin for 15 minutes at room temperature and then centrifuged for a further 15 minutes. The final deposit was also

inoculated on to two slopes of media. All cultures were incubated at 37° C. and examined at intervals for three months. The pH of the contents of the bottles was estimated periodically and the temperature of the room taken. The experiment was continued for nine months and a final test made after 13 months. The results are given in Table I and for the sake of simplicity the average figures are given for the pH of the individual bottles and the maximum and minimum temperature variations only for each month.

The data given in the table show that Johne's bacilli were recovered after nine months from one of each of the series of bottles and not

TABLE I

RECOVERY OF JOHNE'S BACILLI FROM DIFFERENT KINDS OF STERILISED WATER TO WHICH CULTURES HAD BEEN ADDED

Period of Exposure (Months)	*Distilled Water* — *Bacilli Added (mg. per 100 c.c.)* 3·4	1·28	0·1	pH	*Tap Water* — *Bacilli Added (mg. per 100 c.c.)* 3·4	1·28	0·1	pH	*Pond Water* — *Bacilli Added (mg. per 100 c.c.)* 3·4	1·28	0·1	pH	*Room Temperature Range.* ° F.
0	+	+	+	6·8	+	+	+	8·0	+	+	+	5·5	·
1	+	+	+	6·8	+	+	+	7·4	+	+	+	5·3	56–62
2	+	+	+	6·8	+	+	+	7·1	+	—	+	5·3	49–65
3	+	+	+	6·6	C	+	+	7·5	+	+	+	5·3	54–59
4	+	+	+	6·6	+	+	+	7·6	+	+	+	5·8	55–64
5	+	+	+	6·6	+	+	+	7·8	+	+	+	5·6	53–63
6	+	+	—	6·4	+	+	+	7·6	+	+	+	5·9	58–68
7	+	+	—	6·4	+	+	+	7·6	+	+	+	5·4	57–71
8	+	+	—	6·4	+	+	+	7·7	+	—	+	5·4	61–79
9	—	+	—	6·4	+	—	—	7·6	+	—	—	5·5	61–70
13	—	—	—	5·6	—	—	—	6·9	—	·	—	5·1	·

\+ = Growth of Johne's bacilli.
— = No growth.
· = No examination made.
C = Tubes contaminated.

after 13 months. Under the conditions of the experiment there is no apparent difference between the survival times in sterilised distilled water, tap water, and pond water containing dried mud. A profuse growth was obtained at the beginning of the experiment and there was a marked reduction after the first month and a gradual decrease in the number of colonies developing in succeeding months. Towards the end, the number of colonies on the slopes was less than 40 and often less than ten ; this points to a progressive diminution in the infectivity of the water. Although duplicate samples were subjected to antiformin the evidence is insufficient to show whether this treatment inhibited the development of growth. During the period of survival the pH of the waters varied between 6·4 and 6·8 for the distilled water, 7·1 and 8·0 for the tap water

and 5·3 and 5·9 for the pond water. The temperature of the room in which the bottles were kept fluctuated during the time between 49° and 79° F.

(B) SURVIVAL OF JOHNE'S BACILLI IN NATURALLY INFECTED INTESTINAL SCRAPINGS IN UNSTERILISED RIVER WATER IN THE OPEN AIR

A further experiment was planned to test the survival of Johne's bacilli when scrapings of infected bovine intestines were added to river or ditch water. Preliminary tests were made to determine the strength of antiformin and the time of exposure necessary to destroy the bacteria normally present in such water. Samples of water were taken from ditches subject to flooding by the River Thames and exposed to varying strengths of antiformin for different times ; the results showed that exposure to 15 per cent. for 30 and 45 minutes and 20 per cent. for 20 and 30 minutes were satisfactory for the purpose in view. No added advantage was apparent by using 10 per cent. sulphuric acid for 10 to 30 minutes.

Two main experiments were made :—

Experiment 1.—Two Winchester Quart bottles were filled with flood water from the River Thames taken from meadows on which cattle had grazed ; none of the cattle was infected with tubercle bacilli, Johne's bacilli, or *Brucella abortus*. A suspension of intestinal scrapings from a bovine infected with Johne's disease was prepared by making up 6·55 g. of moist scrapings to 70 c.c. with saline. To each bottle of water was added 35 c.c. of suspension ; the bottles were then shaken and their necks covered with one thickness of butter muslin. They were then placed in the shade on the north side of the house and two samples each containing 50 c.c. were removed at approximately monthly intervals. The samples were centrifuged and the deposits treated with 10 c.c. of 15 per cent. antiformin for 30 and 45 minutes and 10 c.c. of 20 per cent. for 20 and 30 minutes. The mixtures were then diluted with eight volumes of sterile saline and centrifuged and the deposits inoculated on to slopes of the modified Dunkin's medium with capillary pipettes. After incubation at 37° C. selected cultures were tested for their morphology and staining reactions, their ability to grow on the modified Dunkin's medium and their inability to develop on egg medium, 5 per cent. glycerine egg medium and agar. The results of the experiment are given in Table II.

The experiment began in January and, as shown in the table, Johne's bacilli were recovered after 113 days' exposure. The early period of survival coincided with severe weather and between the first and the 30th day of exposure the water was frozen and the samples were taken after the ice had been gradually melted by placing the bottles in an unheated outhouse. They were then returned to their place in the open.

A subsidiary test was made at the same time with cultures of

Johne's bacilli. A rich living suspension was prepared from cultures by emulsifying 0·431 g. of bacilli in 10 c.c. of saline. To each of two Winchester Quarts containing similar flood water 5 c.c. of this suspension was added. Johne's bacilli were recovered after one day's exposure but not after 30 days or at any subsequent time. No explanation can be given for this short period of survival.

TABLE II

RECOVERY OF JOHNE'S BACILLI FROM FLOOD WATER CONTAMINATED WITH INTESTINAL SCRAPINGS FROM A CASE OF JOHNE'S DISEASE

Date	Period of Exposure (Days)	*Antiformin Treatment*			
		15 *per cent.*		20 *per cent.*	
		30 *mins.*	45 *mins.*	20 *mins.*	30 *mins.*
29.1.41	1	+++	+++	+	C
27.2.41	30	+++	+++	+	++
26.3.41	57	C	++	C	++
22.4.41	84	—	—	—	+
21.5.41	113	—	+	+	—
18.6.41	141	—	—	C	—
17.7.41	170	—	—	—	—

+++ ++ + = Approximate amount of growth of Johne's bacilli.
— = No growth.
C = Tubes contaminated.

Experiment 2.—In the second experiment similar intestinal scrapings from another case of Johne's disease were mixed with river water and placed in two shallow porous earthenware bowels. The bowls were about 20 inches in diameter and 4 inches deep and both were placed in the open air, one in the shade and one in a spot exposed to sunshine. Samples, increasing in volume from 10 to 80 c.c., were removed at intervals. A similar technique was used for the isolation of the bacilli except that in this experiment the deposits of centrifuged samples were exposed to 10, 15 and 20 per cent. of antiformin for 30 minutes only. As before, cultures from each sample were examined for their morphology and cultural characteristics before being accepted as Johne's bacilli. During the period of the experiment a record of the atmospheric temperatures was made. The results are given in Table III.

This experiment began at the latter end of September and Johne's bacilli were recovered from the bowl kept in the shade after 135 days but not after 163 days. From the bowl kept in the sunny area bacilli were isolated after 163 days and not after 218 days; the last positive culture in this series consisted of one colony only, there having been a gradual diminution in the richness of the growth. During the period of survival the atmospheric temperatures varied between 20° and 64° F. and during the very cold weather the

material was often frozen ; it was never dry and the actual amount of the mixture varied with the rainfall.

TABLE III

RECOVERY OF JOHNE'S BACILLI FROM RIVER WATER CONTAMINATED WITH INTESTINAL SCRAPINGS FROM A CASE OF JOHNE'S DISEASE

Date	*Period of Exposure (Days)*	*In Shade* — *Antiformin Percentage*			*In Sunshine* — *Antiformin Percentage*			*Atmospheric Temperature Range* ° F. *
		10	15	20	10	15	20	
14.10.41	21	+	·	+	+	·	+	·
28.10.41	35	+	·	+	+	·	+	32–64
27.11.41	67	+	·	+	+	·	+	28–64
31.12.41	99	C	·	+	C	·	+	24–54
5. 2.42	135	C	·	+	C	·	+	20–47
4. 3.42	163	—	·	—	·	+	—	20–48
28. 4.42	218	C	·	—	·	—	—	26–62
27. 5.42	247	·	—	—	·	—	—	38–68
22. 6.42	273	·	—	—	·	·	·	42–80
3. 7.42	284	·	·	·	—	—	—	44–78

\+ = Growth of Johne's bacilli.
— = No growth.
C = Tubes contaminated.
· = No examination made
* = During period since previous date of examination.

(C) SURVIVAL OF JOHNE'S BACILLI IN NATURALLY INFECTED FAECES EXPOSED TO ATMOSPHERIC CONDITIONS

A final series of tests was made with faeces from natural cases of Johne's disease. These tests were designed to imitate natural circumstances as far as possible.

Experiment 1.—Fluid faeces were collected from a natural case of disease and placed in a shallow porous bowl similar in size to those already used. This was placed in the open air exposed to the full effect of sunshine and rain. It was not interfered with in any way other than being stirred at the time of sampling. Examinations were made at intervals, and the size of the samples varied, increasing in volume towards the end of the experiment. The contents of the bowl were sometimes fluid, sometimes frozen, and at times dried by the sun. The treatment of samples with antiformin and the technique of isolation were similar to those used in former experiments. The results are given in Table IV and Chart 1, and, as before, cultures were tested for morphological, staining and cultural characteristics.

This experiment began in October and under the conditions described Johne's bacilli were recovered after 246 days during which time the faeces had been frozen and dried and exposed to atmospheric temperatures varying between 26° and 74° F.

TABLE IV

RECOVERY OF JOHNE'S BACILLI FROM NATURALLY INFECTED FAECES EXPOSED TO NATURAL CONDITIONS IN A SHALLOW POROUS BOWL

Date	*Period of Exposure (Days)*	*Result*	*Condition of Contents of Bowl*	*Atmospheric Temperature Range ° F.* *	*Rainfall (Inches)* *
2.11.42	7	+	Fluid	32–50	2·81
24.12.42	59	+	Sometimes completely and	26–53	6·64
25. 1.43	91	+	sometimes partially	28–51	6·76
27. 2.43	124	+	frozen	30–56	5·68
29. 3.43	154	+		27–54	0·57
20. 4.43	176	+	Nearly dry	34–70	0·07
29. 5.43	215	+	Nearly dry	33–72	2·95
29. 6.43	246	+	Dry	40–74	1·65
6. 8.43	284	—	Fluid	46–86	2·17
4. 9.43	313	—	Fluid	45–76	1·67
1.10.43	340	—	Slightly fluid	33–71	1·06

+ = Growth of Johne's bacilli.
— = No growth of Johne's bacilli.
* = During period since previous date of examination.

CHART I

Recovery of Johne's bacilli from naturally infected faeces.
(Tables IV and V.)

+ = Recovery of Johne's bacilli. — = Johne's bacilli not recovered.
A from porous bowl (Table IV). B from porous bowl (Table V). C from enamel bowl (Table V).
Rainfall is shown in inches per four weeks and the maximum and minimum atmospheric temperatures are plotted for each week.

Experiment 2.—Another experiment of a similar kind was made with a fresh sample of infected faeces. A portion was exposed in a shallow porous bowl and some in an enamel bowl; this latter

bowl was not allowed to dry and on one occasion rain water was added. Samples were taken at approximately monthly intervals and the quantities examined increased in volume towards the end of the test. As on other occasions cultures were tested for their morphological, staining and cultural characteristics. The result of the test is given in Table V and Chart 1.

TABLE V

RECOVERY OF JOHNE'S BACILLI FROM NATURALLY INFECTED FAECES EXPOSED TO NATURAL WEATHER CONDITIONS

Date	*Period of Exposure*	*Porous Bowl*		*Enamel Bowl*	*Atmospheric Temperature Range* ° *F.* *	*Rainfall (Inches)* *
		Result	*Condition of Contents*	*Result*		
7.12.42	3	+	Fluid	·	35–50	0
4. 1.43	31	+	Frozen	·	28–53	4·85
4. 2.43	62	+	Fluid	·	28–51	8·32
3. 3.43	89	+	Fluid	·	28–56	2·76
5. 4.43	122	·	Dry	+	27–57	0·61
20. 4.43	137	+	Dry	·	34–70	0·43
5. 5.43	152	+	Dry	+	34–57	0·97
2. 6.43	180	—	Fluid	+	33–74	2·63
30. 6.43	208	—	Dry	+	40–74	1·08
28. 7.43	236	—	Fluid	—	46–74	1·62
25. 8.43	264	—	Fluid	—	45–86	1·30
23. 9.43	293	—	Fluid	—	37–71	1·78

\+ = Growth of Johne's bacilli.
— = No growth.
· = No examination made.
* = During period since previous date of examination.

Under these conditions Johne's bacilli were viable in the porous bowl after 152 days; the rainfall for about seven weeks before this final positive result was low, the atmospheric temperature had risen to 70° F. and the contents of the bowl had become dry during the latter part of the survival period. The faeces in the enamel bowl were not allowed to become dry and on one occasion (April 14th, 1943) the bowl was filled with rain water; Johne's bacilli were recovered from this source after 208 days. The temperature fluctuated between 27° and 74° F. during the period of survival.

Some subsidiary tests were made with other samples of infected faeces and the brief details are given.

Experiment 3.—Some infected bovine faeces had been placed in a flat enamel bowl on September 23rd, 1941, and were allowed to dry. Johne's bacilli were cultured from samples taken on December 5th, 1941, and March 5th, 1942, but not from samples taken on April 7th, 1942, and at subsequent dates. Johne's bacilli had therefore remained viable in this case for 163 days or approximately 23 weeks.

Experiment 4.—A further sample of infected bovine faeces was

examined on the day it was exposed in an enamel tray. Johne's bacilli were recovered on that day (December 21st, 1942) but were not isolated from a sample examined five months later (May 29th, 1943).

Experiment 5.—The faeces of a goat which had been artificially infected with Johne's disease were exposed to the open air in an enamel bowl on October 26th, 1942. Johne's bacilli were recovered from a sample examined that day and from a sample examined on January 1st, 1943, but not from a sample examined on April 20th, 1943. The Johne's bacilli had therefore survived in this experiment for at least 67 days.

DISCUSSION

The main experiments concern the addition of infected intestinal scrapings to river and ditch water and the exposure of naturally infected bovine faeces to atmospheric variations. This latter series approximates to the conditions obtaining in nature and the crucial data are given graphically (Chart 1). It shows that Johne's bacilli in naturally infected faeces survived from October to June and in another case from December till the last day of June. During the periods of viability the volume of faeces increased and decreased with rainfall and evaporation; at times the mixtures were frozen and after exposure to sunshine the material became dry. The survival times are obviously influenced by the intensity of the infection in faeces and by these seasonal factors. Variation in the moisture content is probably of great importance and in one trial (C, Experiment 2) Johne's bacilli were viable for a longer time in the duplicate sample which was kept moist than in the one allowed to dry. The variability of all the factors is probably greater under absolutely natural conditions than in these experiments and this is especially so with the washing effect of rain. Our observations, however, give support to certain suggestions made in the control of Johne's disease. It is not unreasonable, considering the longevity of the organism, to allow infective pasture to be regarded as unsafe for at least one year (Edwards, 1938). It is also a sound policy to encourage such methods of procedure as the breaking up of manure on infected pasture land, the disposal of manure from standings to cultivated land and the draining or fencing of ponds (Dunkin 1929, Minett 1933).

SUMMARY

(1) Cultures of Johne's bacilli remain alive in sterilised water for upwards of nine months.

(2) Intestinal scrapings from cases of Johne's disease may contain viable Johne's bacilli for upwards of 163 days after mixing with unsterilised river water.

(3) Naturally infected faeces, when left exposed to atmospheric conditions, may contain viable Johne's bacilli as long as 246 days

after exposure, and these findings have been correlated with the rainfall and atmospheric temperatures.

(4) The practical application of these results to the control of Johne's disease has been discussed.

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